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Technologies and Management Strategies for Hazardous Waste Control

Volume II—Working Papers

Part B

Application of Biotechnology to
Hazardous Waste Disposal

April 1983

CONGRESS OF THE UNITED STATES
Office of Technology Assessment
Washington, D. C. 20510

Preface

This volume contains papers written for OTA to assist in preparation of the report *Technologies and Management Strategies for Hazardous Waste Control.* OTA does not endorse these papers. In several instances, the OTA report reaches somewhat different conclusions because of additional information which was obtained later. These papers, however, may prove valuable for readers needing more detailed or specific information than could be accommodated in the final assessment report, and are being made available for such purposes.

American Technology Management Consultants

FINAL REPORT

APPLICATION OF BIOTECHNOLOGY
TO
HAZARDOUS WASTE DISPOSAL

JANUARY 28, 1982

SUBMITTED TO

MATERIALS PROGRAM
OFFICE OF TECHNOLOGY ASSESSMENT
UNITED STATES CONGRESS
WASHINGTON, D.C. 20510
ATTN DR. SUELLEN PIRAGES

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TABLE OF CONTENTS

Page

LIST OF TABLES v

LIST OF FIGURES vii

EXECUTIVE SUMMARY viii

I. INTRODUCTION 1

II. POTENTIAL APPLICATION OF BIODEGRADATION PRINCIPLES TO HAZARDOUS WASTES 2

- A. Methods for Manipulating System Biology 3
  - 1. Mutation and Selection (Genetic Engineering) 3
  - 2. Plasmid Evolution 8
  - 3. Substrate Specific Enzyme Detoxification . . 9
  - 4. Bioaugmentation 9
- B. Factors that Affect the Ability to Biodegrade Hazardous Materials 11
  - 1. Biological 11
  - 2. Chemical 15
  - 3. Physical 19

REFERENCES II-1

III. BIOLOGICAL TECHNOLOGIES CURRENTLY AVAILABLE 21

- A. Biotechnology Descriptions 21
  - 1. Conventional Biological Treatment 21
  - 2. Land Disposal 32
  - 3. Enzyme Detoxification 37
  - 4. Specialized Fermentations 38
  - 5. Spill Cleanup Systems 39
- B. Biotechnology Costs and Comparisons to Alternatives 39
- C. Problems and Advantages of Biological Technologies 46
  - 1. Operational Factors 47
  - 2. Advantages 52
  - 3. Public Health Risks 54

REFERENCES III-1

TABLE OF CONTENTS
(CONTINUED)

Page

IV. BIOTECHNOLOGY APPLICATIONS IN SELECTED INDUSTRIES . . 61
- A. Electroplating 62
- B. Pesticides 62
- C. Leather Tanning 65
- D. Chlorinated Hydrocarbons 65
- E. Toxic Material Spills 66
- F. Hazardous Waste Remedial Cleanup 69
- G. Pretreatment Technologies 70
- REFERENCES IV-1

V. MARKET ANALYSIS 73
- A. Biotechnology in Relation to the Total Hazardous Waste Market 73
- B. Market Profile and Potential 75
  - 1. Manufacturers and Suppliers of Equipment and Materials 77
  - 2. Architectural and Consulting Engineers . . . 85
  - 3. Operating Companies 87
- REFERENCES V-1

VI. BIOTECHNOLOGY AS A MAJOR HAZARDOUS WASTE TREATMENT TECHNOLOGY-FACTORS AFFECTING DEVELOPMENT 90
- A. Commercial Application and Research and Development Pontential 90
  - 1. Potential of Biotechnology as a Primary Treatment Method 90
  - 2. Research and Development 102
- B. Legislative and Institutional 115
  - 1. Legislations and Regulations 115
  - 2. Institutional Barriers 120
- C. Scenarios 126
- REFERENCES VI-1

APPENDIX 129

LIST OF TABLES

Table		Page
S-1	SUMMARY OF THE APPLICATIONS OF BIOLOGICAL TECHNOLOGY TO HAZARDOUS WASTE	x
II-1	METHODS FOR MANIPULATING SYSTEM BIOLOGY .	4
II-2	FACTORS CONTRIBUTING TO THE RECALCITRANCE OF CHEMICALS AND POTENTIAL BIOLOGICAL MANIPULATION METHODS	12
II-3	SELECTED ORGANIC CHEMICALS THAT CAN BE DEGRADED BY BLENDED SPECIALIZED BACTERIAL SEED CULTURES	16
II-4	BIODEGRADATION POTENTIAL OF DILUTE TOXIC WASTES	17
II-5	TOXIC POLLUTANTS REPORTED TO CONCENTRATE IN SLUDGE	20
III-1	SUMMARY OF BIOTECHNOLOGIES TO TREAT HAZARDOUS WASTE	22
III-2	SUMMARY OF COSTS FOR ALTERNATIVE PHENOLIC WASTEWATER TREATMENT SYSTEMS	40
III-3	APPROXIMATION OF COST PER POUND ORGANIC FOR EXISTING BIOLOGICAL TECHNOLOGIES . . .	43
III-4	MAJOR OPERATIONAL CONCERNS IN HAZARDOUS WASTE TREATMENT WITH BIOTECHNOLOGIES . .	47
III-5	ADVANTAGES OF BIOTECHNOLOGIES	53
III-6	PUBLIC HEALTH RISKS ASSOCIATED WITH THE USE OF BIOTECHNOLOGIES IN HAZARDOUS WASTE CONTROL	55
IV-1	REMOVAL OF CHLORINATED HYDROCARBONS REPORTED FOR AEROBIC SYSTEMS	67
V-1	QUANTITY OF HAZARDOUS WASTE GENERATED BY MAJOR SECTOR	75
V-2	TYPES OF PRODUCTS IN THE BIOLOGICAL TREATMENT OF HAZARDOUS WASTE	78

LIST OF TABLES
(CONTINUED)

Table		Page
V- 3	ESTIMATE OF 1981 CAPITAL EXPENDITURE FOR BIOTECHNOLOGIES IN MANUFACTURING SECTOR .	79
V- 4	COMPANIES ENGAGED IN SALES OF BIOLOGICAL FORMULATIONS OR GENETIC ENGINEERING FOR HAZARDOUS WASTE	81
V- 5	ESTIMATED PLANT EQUIPMENT MARKET POTENTIAL FOR BIOTECHNOLOGY IN HAZARDOUS WASTE TREATMENT	83
V- 6	MARKET POTENTIAL OF BIOLOGICAL FORMULATIONS FOR HAZARDOUS WASTES TREATMENT	86
V- 7	ESTIMATED SALES FOR CONSULTING ENGINEERS IN BIOTECHNOLOGY	85
V- 8	MAJOR HAZARDOUS WASTE MANAGEMENT FIRMS .	87
V- 9	ESTIMATED CURRENT ANNUAL COSTS (1981) OF BIOTECHNOLOGY APPLICATIONS IN HAZARDOUS/TOXIC MATERIALS CONTROL	89
VI-1	POTENTIAL APPLICATIONS OF BIOTECHNOLOGY FOR HAZARDOUS WASTE TREATMENT	91
VI-2	COMPARISON OF COST FOR LANDFILLING ETHYLENE DICHLORIDE/VINYL CHLORIDE WASTE TO ANAEROBIC FERMENTATION	97
VI-3	MAJOR GOVERNMENT PRIVATE CONTRACTORS AND IN RCRA-C RELATED BIOLOGICAL TECHNOLOGY .	104
VI-4	ENVIRONMENTAL PROBLEMS POTENTIALLY SOLVABLE BY APPLIED GENETICS	106
VI-5	MAJOR GOVERNMENT RESEARCH AND DEVELOPMENT FOR BIOLOGICAL TECHNOLOGIES IN CONTROL OF HAZARDOUS WASTE	109
VI-6	INSTITUTIONAL FACTORS THAT AFFECT THE DEVELOPMENT OF BIOLOGICAL TECHNOLOGIES IN HAZARDOUS WASTE CONTROL	121

LIST OF FIGURES

Page

II-1 TYPES OF GENETIC ENGINEERING TECHNIQUES . . 7

III-1 HYPOTHETICAL RESPONSE OF THE MICROBIAL POPULATION FOLLOWING MULTIPLE APPLICATIONS OF AN ORGANIC CHEMICAL TO SOIL 34

III-2 COMPARISON OF LIFE CYCLE COSTS FOR CONVENTIONAL BIOLOGICAL TREATMENT TECHNOLOGY 41

III-3 COST/TECHNOLOGY/CONCENTRATION RELATION IN HAZARDOUS WASTE TREATMENT 45

IV-1 PROPOSED BIOLOGICAL TREATMENT FACILITY FOR 2,4-D and 2,4,5-T 64

IV-2 TOP VIEW OF TYPICAL PROCESS FLOW CHART FOR SPILL CLEAN UP 68

IV-3 OXYPHOTON PROCESS 71

V-1 ESTIMATED BIOTECHNOLOGY EXPENDITURES FOR 1981 IN TREATING TOXIC WASTES FROM THE MANUFACTURING SECTOR 74

VI-1 CURRENT HAZARDOUS WASTE MANAGEMENT AND WITH BIOLOGICAL TECHNOLOGY 101

EXECUTIVE SUMMARY

Biological technologies have been used by civilizations for thousands of years in the agricultural, food, and pollution control fields. Recent developments in applied genetics, or the ability to manipulate the genetic structure of primitive and higher cells, has brought the ability to expand this horizon and to enter into new fields that a decade ago were considered highly remote.

One of the most serious problems confronting our industry and the environment today is the disposition of the large quantity of hazardous and toxic materials generated and discarded by manufacturing processes. It is estimated by the Environmental Protection Agency that there are nearly 50 million metric tons of hazardous waste generated each year, a sizable majority of which are toxic mixtures of little commercial value. Many of these are organic chemicals and are considered as carcinogens at significant exposures. Existing destruction and disposal technologies are either inadequate as a long term solution or expensive. Some industries place the annual cost of current regulations between $10-50 billion. Costs of landfilling highly hazardous waste requires appropriate financing to assure near perpetual care to guard against long term liability risks. Existing destruction techniques can be exorbitant in cost. For example, a recent commercial incineration required at sea due to toxicity, cost nearly $8 a gallon, nearly one-fourth the cost of a barrel of crude oil. Many of these chemicals are, however, potentially biodegradable given the right biological, chemical, and physical conditions. When these conditions can be met, biological methods are the prevailing treatment practice for toxic wastes as it is cost-effective.

In this study for the United States Congress, Office of Technology Assessments, AmTech Consultants has explored the potential of biotechnology to treat hazardous wastes, considering the applications of various biological manipulation techniques. We have reviewed available technologies and their costs, problems and advantages and have presented various emerging approaches. Likewise, we have conducted a market analysis and the projection of sales to 1990 as well as an assessment of the legislative, institutional, health, and other factors to bear. These are summarized in Table S-1. Several hundred individuals were contacted in government, industry, and academia and their opinions as well as scientific reports and government publications are presented in this technology assessment.

Though biological technologies have been in use for several decades to treat industrial wastes more attention has been given to the design of engineering hardware rather than the biology of the system. Recent advances in industrial microbiology can now allow more attention to the biology. Biological manipulation techniques considered in this study are genetic engineering, plasmid evolution, substrate specific enzyme detoxification, and bioaugmentation. Genetic engineering makes use of any one of a number of techniques, both natural and induced, to alter the genetic structure of microorganisms and to select those that are more able to efficiently biodegrade the toxic waste. Plasmid evolution is analagous to genetic engineering but is a process that occurs in nature whereby microorganisms exchange extrachromosomal genetic material to select for resistance against materials toxic to the cell. Through prudent selection of various microorganisms this natural evolutionary process can be controlled in the laboratory with microorganisms resulting that are highly resistant to the toxic material in question.

Table S-1

SUMMARY OF THE APPLICATIONS OF BIOLOGICAL TECHNOLOGY TO HAZARDOUS WASTE

A. METHODS TO MANIPULATE THE BIOLOGY OF WASTE TREATMENT SYSTEMS

a. Genetic Engineering - laboratory technique

b. Plasmid Evolution - natural process

c. Substrate Specific Enzyme Detoxification

d. Bioaugmentation - field application

B. BIOLOGICAL TECHNOLOGIES

AVAILABLE

a. Conventional Aerobic and Anaerobic Waste Treatment Systems (reactors/ponds)

b. Land Based Systems (landfarming/compost)

EMERGING

c. Enzyme Detoxification

d. Specialized Fermentations

e. Pretreatment Techniques

C. COST PER MASS DESTROYED

a. anaerobic $<$ landfarming $<$ aerobic

D. PROBLEMS AND ADVANTAGES

a. Biotechnology is waste specific (can't be used on all wastes)

b. Cell lines need development/kinetics

c. Destroys waste by using as food

d. Low risk/low containment

5

Table S-1 (CONTINUED)

E. MARKET ANALYSIS

- a. 1981-Products \$1-2 billion
- b. 1990-Products \$5-10 billion

F. POTENTIAL COMMERCIAL APPLICATIONS

- a. RCRA-C listing reviewed
- b. Fermentation example provided
- c. Public research in planning
- d. Private research proprietary

G. LEGISLATIVE AND INSTITUTIONAL

- a. Permitting and standards could be detrimental to on-site/new applications
- b. Professional attitudes
- c. Possible lack of skills

H. SCENARIOS FOR DEVELOPMENT

- a. Liability of waste provides incentive/ enforcement not too critical/national need
- b. Technical achievements by industry and government principal factor/R&D
- c. Strong economy best
- d. Lack of developments could lead to mid-term imports

This resistivity is due to detoxification reactions within the cell that can allow the microorganisms to then use the material as a source of food. Through either genetic engineering or plasmid evolution more efficient microorganisms can be obtained for commercial use in biodegrading or attenuating toxic waste. In each of the above instance it is enzymes that are being coded for by DNA that are responsible. These enzymes can be extracted from the cells and used as catalysts in a free form or as attached to materials such as glass beads to enhance the rate of detoxification in reactors or the open environment. One enzyme usually only catalyzes one reaction and is often specific to a waste or chemical structure and is termed substrate specific enzyme detoxification. The use of the whole living cell containing not one but many specific enzymes obtained from genetic engineering or plasmid evolution, is termed bioaugmentation, as it increases the biological capacity of the waste treatment technology to destroy toxic materials.

Existing biological technologies applied to toxic waste treatment are conventional aerobic and anaerobic systems as developed for water pollution control. Land farming of solid waste is also used as a means of biodegradation and variations thereof are frequently the only practical means to handle spills of toxic materials or decontaminating hazardous waste dumps. Emerging technologies are considered to be the use of free or immobilized enzymes, fermentation systems similar to those currently used in the pharmaceutical industry, and physical/chemical pretreatments that allow for less biological recalcitrance of the treated material.

Aerobic systems are the most expensive as they are used to treat relatively dilute waste streams and large amounts of water have to be accomodated. As these streams become more concentrated in organic materials, oxygen has to be mechanically introduced to supply air to the bacteria and can be energy intensive and expensive. Anaerobic systems are the least expensive on a per mass destroyed basis as they do not require oxygen. They can also produce methane as a by-product. Anaerobic systems are sensitive and require close operating supervision, however. Land farming is usually used to destroy the spent bacteria that have grown on the waste and frequently contain residuals of toxic materials.

Biotechnologies are specific to waste types and changes in concentrations or characteristics can upset a system. Concentration of the waste is not only important due to toxicity effects but also on the choice and cost-effectiveness of technologies. Water is an essential medium in biological systems and aerobic processes are most efficient in dilute concentrations, anaerobic in relatively high (from 90-99% water) and land farming when the material can be handled as a semi-solid sludge. Water, however, is considered a costly burden in landfills or incineration and much effort is spent to physically remove it prior to treatment by these non-biological techniques. Consequently, biological systems can be highly cost-effective when the waste contains appreciable quantities of water. It is also much cheaper to add water than remove it, and consequently, highly concentrated materials could also be biologically treated through dilution.

Because most work on biological manipulation techniniques have focused on plasmid evolution, a process that occurs in nature, it is doubtful that the use of any cell lines will pose no greater risk than applications of natural biological formulations in other industries. Furthermore, the use of any bacteria will be applied in the most practical environment, a hazardous waste treatment facility. Finally, it is probable that in most instances the toxic waste that the organism is degrading poses a much greater risk. Therefore risk and containment concerns are minimum, though screening is suggested for pathogen contamination or toxin production.

The current market for the biological treatment of hazardous waste is estimated to be from $2-3 billion per year, primarily applied on industrial sites for the treatment of industrial wastewaters. For 1990 a reasonable market for product sales is estimated from $5-10 billion, mostly on-site, though there may be more off-site use with pretreatment technologies or in select cases where specific waste is centrally pooled or admixture with other wastes are required.

EPA's current listing of hazardous waste is presented and an estimate of the potential for biological treatment is made. An example is provided for a concentrated still bottom using anaerobic fermentation and is costed in comparison to current methods of disposal. One of the primary reasons that this technique as illustrated is not applied is the lack of research to develop a cell line. When cell lines are developed, it is necessary to establish the cellular kinetic coefficients for system design.

To develop a viable biological technology over the next decade, many legislative/regulatory factors have to be taken into consideration as well as institutional barriers. Regulations developed under the Resource Conservation and Recovery Act may inhibit the development and commercialization of biotechnologies in their current form by discouraging new on-site developments through a complex permitting process. It encourages the upgrading of existing facilities which could assist biotechnologies, but for the most part provides more incentive to provide the waste to a off-site disposer who in turn is reluctant to use biotechnology due to variability in wastes received at facilities (biological technologies require a fairly consistent quantity and quality of waste and there is currently no way to assure this supply cost-effectively off-site).

A key institutional barrier appears to be in professional attitudes. On the one hand engineers have little practical experience in biology, and on the other, biologists have little in engineering. Each profession wants to profit from the new techniques but they must work hand in hand to be successful. In addition, hazardous waste is regulated under a solid waste program for a solid waste industry that has traditionally used transport and landfill as its infrastructure.

When considering the influence of various regulatory, economic, and technical factors on the development of biotechnology as applied to hazardous waste, technical achievement in both the public and private sector prevail. These are stimulated by a strong economy. Though regulations might appear to be a market factor, the real liability the waste poses to industry is sufficient motivation to acquire cost-effective biotechnology even under a passive regulatory enforcement program.

In this regard, investments in research and development by industry is required for applications on their own operations or for use in the commercial market. Government research and development is also important in fostering the basic skills that are necessary and in the conduct of high risk/high reward efforts that industry may be reluctant to undertake on their own. Should an initiative not be taken in the near term to address the national need it is likely that there will be an increase in imports from other countries (e.g. Japan) who have advanced fermentation technologies that may be more cost-effective than current approaches.

I. INTRODUCTION

Nearly all organic chemicals are degraded in the environment to inorganic end products. This degradation occurs naturally through physical/chemical processes such as photooxidation in sunlight or chemical hydrolysis in water, or biologically as in enzyme mediated biochemical reactions. When organic waste is biologically degraded, it serves as a source of food for the growth of cells, tissues, and the synthesis of metabolic byproducts. Microorganisms form the dominant group of organic chemical decomposers in the natural environment, and chief among them are bacteria.

The generation and ultimate disposition of hazardous (toxic) chemicals are major concerns to all industrialized countries and many of the disposal methods currently used are either inadequate or costly. It is estimated that there are nearly 50 million metric tons of hazardous waste generated annually in the United States and many of these are potential carcinogens. A significant fraction of these waste products can be degraded or detoxified biologically by microorganisms.

The purpose of this study is to review the current applications of biotechnology to treat hazardous waste and to assess the potential development of the technology over the next decade. Of particular interest is the role genetic engineering might play in expanding the scope of the current technology base as well as the use of bioaugmentation, that is, the addition of selected microorganisms to particular waste treatment processes to improve degradative performance.

Industrial processes that include the use of biological systems are considered biotechnologies and when applied to waste treatment primarily involve anaerobic and aerobic degradative systems in reactors, lagoons, or on land. These technologies and their general costs, problems, and advantages are reviewed and several examples of their application are provided.

Biological technologies have been used for many years in waste treatment and a fairly mature industry and sizable market has been established for both conventional and toxic pollutants. The expansion of this industry to the hazardous wastes market is evaluated in this study considering existing and new technology developments, and economic/regulatory factors.

The potential for biotechnology to be a major treatment method is considered important for specific types of hazardous wastes and, in this regard, research and development in the public and private sector is reviewed. Legislations and regulations have an influence on the potential development of biological technologies and their impact as well as institutional barriers are explored.

II. POTENTIAL APPLICATION OF BIODEGRADATION PRINCIPLES TO HAZARDOUS WASTE

Many of the chemicals listed in the Environmental Protection Agency's hazardous waste regulations are toxic organics and are potentially biodegradable. When considering whether or not these chemicals can be rendered harmless through biotechnology, many factors specific to the physical and chemical property of the waste, the environment where it is contained, as well as the microbiology of the system need to be taken into consideration. It has been generally assumed as the Principle of Microbial Infallibility, however, that all organic chemicals can be biodegraded under proper conditions. It has also been assumed with the Ubiquity Principle that microorganisms are environmentally diverse and that genetic material is potentially present in the environment for all forms of enzymatically catalyzed organic chemical reactions. These Principles are more often stated than observed due to a wide range of physical, chemical, and biological conditions which can be accounted for as contributing to the recalcitrance of chemical wastes.

The biological factor, or the ability of a microbial population or a specific organism to utilize the waste is of first concern in the application of biotechnology. The term toxicity is not universal to biological cells and in the context of hazardous waste, especially carcinogens, is only applicable to the differentiated and organized cellular tissues of higher plants and animals. Consequently, primitive organisms, such as bacteria and fungi, can be selected to tolerate and metabolize hazardous chemicals as food or substrates and to break down the chemicals through the activity of catabolic enzymes to simple building blocks that in turn form cellular biomass, metabolites, and waste or by-products such as inorganic and organic gases. In mixed cultures co-metabolism can also occur where one organism may incidentally degrade a material while another uses the product as a substrate (Perry, 1979). Biological systems may also actively take up soluble heavy metals or create an external environment for their precipitation to relatively inert chemical forms (Brierley, et al., 1979).

Many basic principles of biochemistry and microbiology are involved and the reader is encouraged to consult general texts to review the fundamentals of cellular metabolism (Lehninger 1971; Stanier et al., 1963; Peppler et al., 1979; Armstrong and Bennett, 1979; Norton, 1981; and Pelczar; 1978).

The microbial processes that degrade organic wastes occur under aerobic and anaerobic conditions. The heterotrophs (organisms that derive carbon and energy from organic compounds) performing these catabolic reactions can be termed either obligate or facultative. Obligate or strict aerobes are those organisms which grow only in the presence of free or atmospheric oxygen, e.g., Bacillus subtilus, and use oxygen as an electron acceptor. On the other hand, obligate or strict anaerobes are those organisms which grow only in the absence of free oxygen, such as members of the genus Clostridium, and some substance other than oxygen serves as a terminal electron acceptor.

Facultative bacteria grow in either case and shift their metabolic pathways accordingly. Facultative bacteria can be represented by Escherischia coli. Respiration generally implies an aerobic process and fermentation an anaerobic process. Aerobic processes always require a supply of oxygen.

Design and operation of system hardware plays a crucial role in the operating efficiency of a biotechnology, but it is the microbiology and the characteristics of the waste that are the factors determining toxic materials destruction. With appropriate system design, manipulation of the system biology may be able to realize the Principles of Ubiquity and Microbial Infallibility for selected hazardous wastes.

A. Methods for Manipulating System Biology

Table II-1 presents methods for manipulating biological systems. Most catabolic reactions, or those involving the destruction of toxic waste, undergo enzyme catalyzed reactions. The biodegradation of a waste is determined by the presence of an active enzyme, its relation to the substrate and their relative concentrations. The complete destruction, or conversion to biomass and ultimately inorganic end products, requires a sequence of enzyme reactions by living cells. Because all enzymes are protein based, they are programmed for by the genetic material of the cell, DNA, and are produced by the genetic mediary, RNA.

DNA is present on chromosomes and plasmids. Plasmids are particularly important in toxic waste degradation as they evolve enzymes to degrade material that is foreign to the cell (Farrell and Chakrabarty, 1979).

By manipulating any of the above factors, specifically the genetic code, plasmids, or particular enzymes, more efficient or new methods of toxic waste treatment can result. Bioaugmentation or the practice of adding cell lines to a treatment process, is the practical application of these biological manipulation techniques.

1. Mutation and Selection (Genetic Engineering)

Organic hazardous waste in still bottoms, sludges, groundwaters, or contaminated soils, can be composed of relatively simple halogenated aliphatics (i.e., dichloroethane) or highly complex substituted ring compounds. Their persistence is primarily due to the inability of the natural environment where they are present as contaminants, to provide the optimum chemical and physical conditions necessary to assist in their biodegradation. Though microbes can be selected which are highly sensitive to chemicals (i.e., mutagenesis in cells for the well known Ames Test), they can also be selected for their high resistance (Chakrabarty, 1981a).

In the Ubiquity Principle it is assumed that microbes are sufficiently diverse in nature and that they contain sufficiently diverse genetic material so that every reasonable innoculum or adapted system should produce the enzymes required for attacking most substrates. This is

Table II-1

METHODS FOR MANIPULATING SYSTEM BIOLOGY

a. Mutation and Selection (Genetic Engineering)

1. Mutation:
    - enhance enzyme production and substrate conformation
    - uses random or controlled (rDNA) processes
    - provides natural or induced techniques
2. Selection:
    - conserves genetic trait
    - uses natural or artificial source techniques
    - requires preservation of cell line

b. Plasmid Evolution:

- promotes degradative enzymes on plasmids
- transmits plasmids between microorganisms
- recruits stable plasmids in one microorganism
- inserts substrate activity on one stable plasmid
- recruits multisubstrate plasmids in one microorganism
- isolates and preserves cell line

c. Substrate Specific Enzyme Detoxification:

- requires cell line identification and extraction of extracellular or intracellular enzyme
- defines substrate specificity and enzyme stability
- detoxifies in single or multiple steps
- applied free or immobilized
- can use immobilized cells

(SOURCE: AmTech Consultants)

Table II-1 (Continued)

METHODS FOR MANIPULATING SYSTEM BIOLOGY

d. Bioaugmentation:

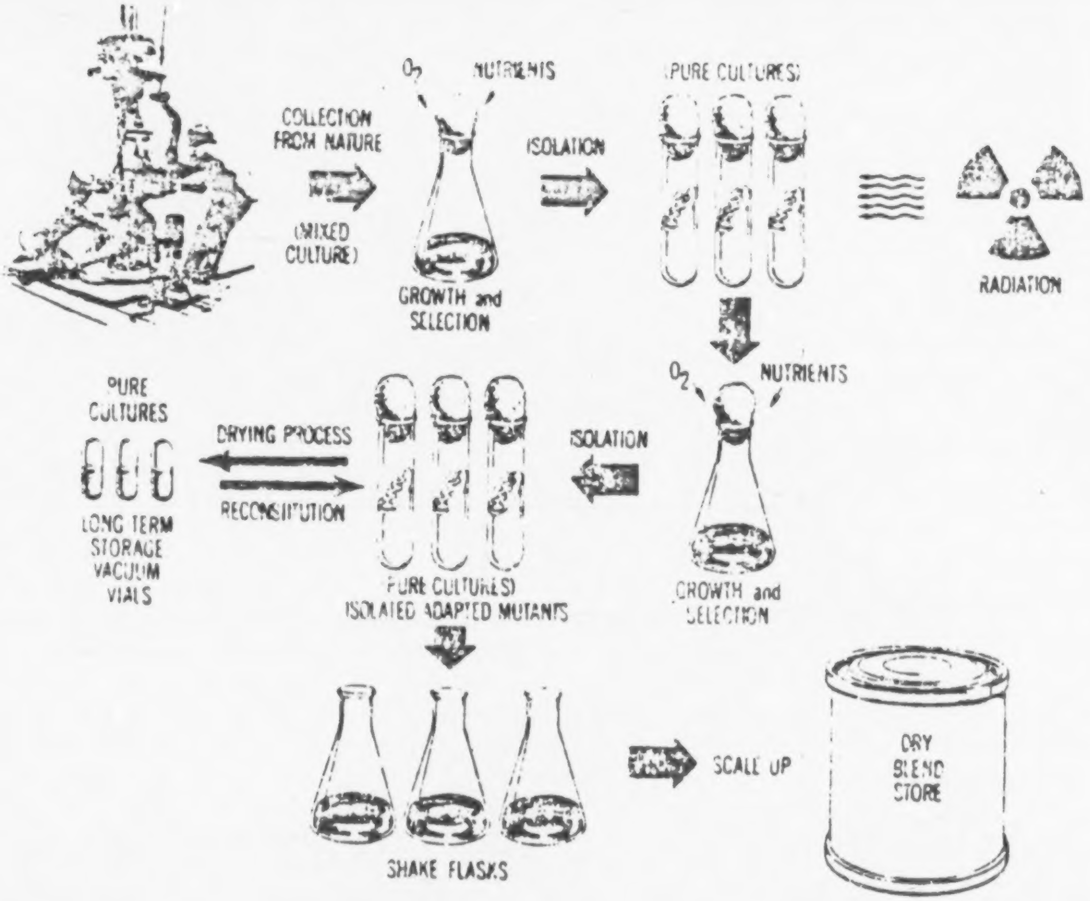
- requires cell line identification and production method
- packages in liquid or dry formulation
- provides substrate activity in single or multiple formulation
- requires method of application
- can replace metabolic deficient systems
- can enhance metabolic performance

(SOURCE: AmTech Consultants)

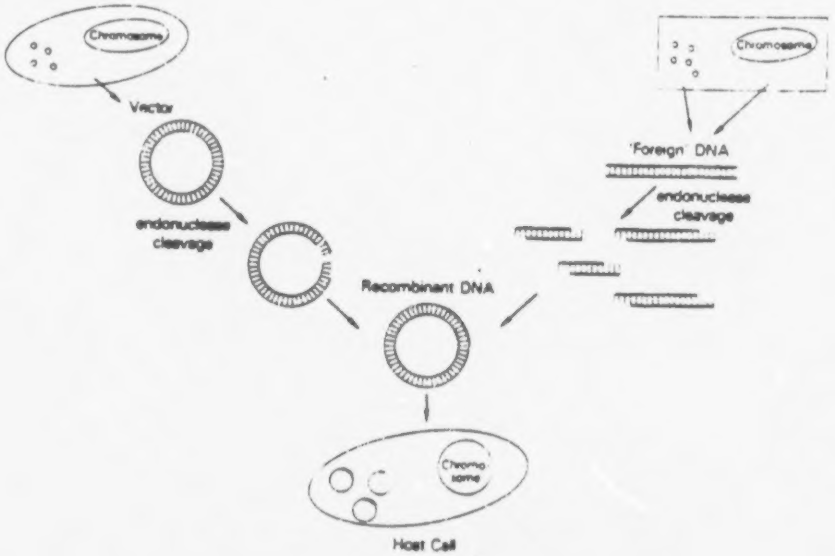
not the case as there are many instances where varying results have been obtained with the same chemical and different types of innocula (Haller, 1978).

Mutations are alterations in the genetic structure of DNA and can result in different amino acid sequences in the coding structures of enzymes and thus effect the enzymes' conformation to a substrate either decreasing, increasing, or allowing for new substrate activity. Mutations in the genetic regions controlling promoter, operator, or regulator DNA sequences can also enhance the rate of transcription of specific proteins by increasing RNA polymerase activity or inhibiting enzyme repression. Ionizing radiation and certain chemicals induce mutations and occur in nature or can be induced in the laboratory. Natural and induced mutations have been commercially used for several decades in the pharmaceutical industry to improve yields of metabolites from producing cell lines (Elander and Chang, 1979). The same principle has also been applied to mutate microorganisms to preferentially degrade specific toxic pollutants (Worne, 1972). Mutations are random processes and natural or artificial selection is required to conserve a beneficial genetic trait. Mutations occur in open environments or treatment systems where toxic waste is present as a contaminant and these sources are frequently used to obtain microbes that have been naturally selected for further refinement in the laboratory (Figure II-1). Artificial selection in the laboratory is usually conducted through enrichment subculturing and plating techniques. Bacteria collected from nature are inoculated into shake flasks and the substrate in question is gradually increased in concentration through serial transfers while other common carbon sources are decreased. Over time, if growth occurs, the microbe(s) dependent on the substrate will be selected over those that are not and are isolated as colonies growing on media plates containing the substrate as a sole source of carbon. The cultures obtained in this fashion are sometimes referred to as a selectively adapted population.

The recombination of genetic material in-vitro (recombinant DNA) or the splicing and insertion of genetic codes into bacterial plasmids, is an induced mutation, and can be considered either randomized, or controlled if the genetic codes are known for a fragment. Recombinant DNA is a potentially powerful genetic engineering tool that allows for the transfer of chromosomal or extrachromosomal DNA from one family, genus, or species to another, so that optimum conditions for the synthesis of a product can be obtained. The real commercial potential for recombinant DNA is transferring a specific genetic sequence from unstable, slowly productive, and highly specialized eukaryotic cells into more stable, rapidly producing, and primitive procaryotic bacterial cells. Because degradative enzymes for toxic materials are usually found on bacterial plasmids (Timmis and Puhler, 1979) and numerous enzymes are involved, the utility of recombinant DNA in toxics degradation may be limited to the exchange of plasmid material between primitive microorganisms (Chakrabarty, 1981a).



Radiation Induced Mutations (Thibault et al., 1979)



Recombinant DNA (Elander et al.,1979)

Figure II-1 TYPES OF GENETIC ENGINEERING TECHNIQUES

For a more thorough description of genetic engineering, the reader should refer to several recent reviews (Science, 1980; Scientific American, 1981;OTA, 1981).

2. Plasmid Evolution

Within the last decade plasmids have been identified and studied for their role in the development of antibiotic resistance in certain strains of infectious microorganisms. More recently, they have also been identified as playing a significant role in the production of catabolic enzymes for specific pollutant degradation (Timmis and Puhler, 1979).

As in the evolution of antibiotic resistance by bacteria, evolution of specific plasmid genetic codes for toxic pollutant degradation occurs. Plasmids are not only hereditary, but also reproduce independently from chromosomal DNA. Plasmids are unique as they are transmissible from one organism to another (Farrell and Chakrabarty, 1979;Pemberton et al., 1979) and between different species, such as between strict aerobes and anaerobes (Kamp and Charkrabarty, 1979). Plasmid interchange occurs naturally in the environment and transfer probabilities have been developed between different types of organisms (Levine, 1981).

The transfer of plasmids between organisms and the manipulation of their DNA "in vitro" can also be performed through recombinant DNA techniques to speed up a natural evolutionary process (Fall et al., 1981). The traditional work in recombinant DNA (Cohen et al., 1973) utilized the natural transfer capacity of plasmids to carry recombined genetic material to bacteria. Taking advantage of the transmissible nature of plasmids, a technique termed Plasmid Assisted Molecular Breeding has been developed to artificially select bacteria that evolve plasmids coding for a specific pollutant's total degradative pathway (Kellogg et al., 1981). Though the technique is similar to routinely used enrichment/selection procedures for isolating cell lines, the knowledge of plasmid evolution allows for a more scientific approach in selecting the initial inocula. In this technique a wide variety of naturally selected bacteria (e.g., from contaminated dump sites) are inoculated with artifically selected bacteria harboring plasmids known to code for various sequences in a degradative pathway. Through subsequent enrichment, subculturing, and plating techniques the objective is to isolate a pure culture that has recruited the plasmids responsible for a waste's total mineralization. The technique can also be referred to as enzyme recruitment (Fall et al., 1979). The technique has been used to obtain a pure cell line capable of totally degrading 2,4,5,-T (Chatterjee et al., 1981) and the cell line has been demonstrated to rapidly degrade highly contaminated soils when the culture is applied to small outdoor test plots (Chakrabarty, 1981b). The objective of plasmid evolution in the laboratory is to produce a functional cell line with a single plasmid encoding total substrate degradation. A single microorganism harboring many plasmids, each plasmid degrading a different substrate, might be considered an ultimate goal to increase the spectrum of mineralization potential.

3. Substrate Specific Enzyme Detoxification

Enzyme catalyzed reactions are responsible for the biodegradation of toxic wastes and many types of reactions have been identified (Alexander, 1981). For the most part these catalyzed reactions fall under the oxidoreductase and hydrolase classifications of the International Enzyme Commission. An enzyme can be highly specific to a substrate or show activity on a broad set of similar functional groups on differing substrates.

Enzymes can be extracted from isolated cell lines and if stable and tolerant to process conditions and not requiring a cofactor can be used in a free or immobilized state to catalyze a one step or possibly two step biochemical reaction. The use of free or immobilized enzymes have received widespread use in industrial processes, for example, in the conversion of starch to sugar, production of amino acids, and synthesis of penicillin. A considerable amount of work has been conducted by Munnecke (1977, 1979) on an immobilized enzyme process that hydrolyzes the phosphorous-phenolic oxygen bond in organophosphorous pesticides. The enzyme, extracted from a cell culture selected for growth on parathion as a sole source of carbon, shows broad substrate specificity on eleven other organophosphorous pesticides.

Microbial enzymes for biodegradation can be classified into two forms, extracellular and intracellular. Intracellular enzymes are expensive to extract from the cell and can be unstable. Due to the loss of free enzymes, immobilized systems, where the enzyme is bound or coated to an inert media (e.g., glass beads, polyacrylamide) and can be retained, are preferred. The general trend in the industry is towards immobilizing viable cells that serve as a solid catalyst and continuously produce enzymes in a fixed or fluidized bed (Chibata et al., 1979). Such systems can also serve multienzyme functions. Extracted enzymes that are stable and have broad substrate specificity can be used to detoxify specific and related hazardous residues (e.g., in pesticide containers, rinse waters, spills).

The extraction and immobilization of many different enzymes on one system has been proposed to increase the spectrum of substrate detoxification capabilities (Munnecke, 1979).

4. Bioaugmentation

The addition of biological materials to assist in the treatment of organic wastes is frequently termed bioaugmentation. Materials added are not only microbes, but can include other additives or biological products to assist in the performance of waste management. Bioaugmentation can be used to pretreat or emulsify waste, aid in the start-up of a biological treatment system, after a biological process has been in operation to overcome treatment difficulties or deficiencies in design, or considered as part of routine operations and maintenance. Bioaugmentation is also used for specialized cleaning in areas that are difficult to routinely maintain, such as the bilge of tankers, or lift stations, and in impoundments and lagoons. The main purpose in their use, however, is to overcome the factors contributing to the persistence of chemical compounds.

When a biological treatment system is initially started, acclimated cultures are usually sought, and for cases where a natural seed is not readily available, bioaugmentation can be used. The propagation of the initial culture takes place over an extended period of time, depending upon the amount of the initial culture used and its adaptation to the substrate. Bioaugmentation can assist in increasing the cell mass of slowly growing microorganisms and maintain populations which normally might be lost from the system (Young, 1979; Nichols, 1978). For example, specific subpopulations growing on a particular substrate or toxic pollutant may have low or deficient growth kinetics resulting in higher than desired concentrations of the waste pollutants. In such cases bioaugmentation can be used as a routine maintenance operation (Tracy et al., 1979; Zitrides, 1978; Thibault, 1978). Bioaugmentation is most frequently used in conventional wastewater treatment, but is finding increased use in the treatment of toxic wastes, such as the cleanup of toxic spills (Walton and Dobbs, 1980; Olivieri et al., 1976).

Many suppliers do not produce their own cultures and the growth process is done by specialty firms. Production reactors can range up to 2,000 gallons, and a unique process, patented by General Environmental Science Corporation produces seven different species of aerobic and facultatively anaerobic organisms in liquid form (Horsfall and Gilbert, 1976). Most other firms market mixed cultures in dry form. This includes Sybron Chemicals, Cytox Corporation, and Flow Laboratories. In the production process, care is taken to prevent contamination, largely from Salmonella, Staphlococcus and Streptococcus. Reactor conditions are controlled to optimize production on substrates of concern, producing well acclimated induced bacteria that are normally separated by centrifugation or filtering. Freeze or air drying is performed prior to packaging. The viability of a microbial spore former is not too severely affected by the freeze drying process but damage to the cell wall of non-spore formers is critical and they are usually air dried slowly. Contents of commercial shipments usually contain the microorganisms and enzymes, nutrients, surfactants, and a filler.

B. Factors that Affect the Ability to Biodegrade Hazardous Wastes

There are many factors that affect the ability to biodegrade hazardous waste and approximately 15 general and interrelated factors can be divided into biological, chemical, and physical categories as adapted from Alexander (1973). These factors are presented in Table II-2 together with some of the biological manipulation methods and other techniques that might be used to overcome their effects.

1. Biological

The biological factors presented in Table II-2 are nearly all based upon the absence of a genetic code or the loss of activity of an enzyme in a biological system. In regard to the non-existence of an active organism both genetic engineering techniques (Fall et al., 1981) and plasmid evolution in nature (Timmis and Puhler, 1979; Farrell and Chakrabarty, 1979; Pemberton et al., 1979; Kamp and Chakrabarty, 1979) can be expected to recruit enzymatic capabilities within microorganisms to biodegrade specific toxic wastes. Cultures have been isolated from Hudson River sediments, for example, capable of degrading polychlorinated biphenyls with up to four chlorine substituents (Kamp and Chakrabarty, 1979) and these cultures can be further manipulated in the laboratory using plasmid evolution or genetic engineering techniques.

As briefly discussed in the previous section, there are a myriad number of reactions that can take place dependent upon the structural form of the substrate. These reactions include the following (Alexander, 1981).

Dehalogenation
Deamination
Decarboxylation
Methy oxidation
Hydroxylation and ketone formation
Beta oxidation
Epoxide formation
Hydrogenation
N oxidation
Sulfur oxidation
Sulfoxide oxidation
Triple bond reduction
Double bond reduction
Double bond hydra.
Nitro metabolism
Oxime metabolism
Nitrile.amide metabolism

The absence of these reactions when the genetic potential is present can be a violation of comparative biochemistry, enzyme specifity or induction.

Enzymes are under genetic and non-genetic control. They can either be induced by the presence of a substrate or a structurally related analogue through an operator gene on the DNA, or repressed in a similar process through a repressor gene. Some of these operator and repressor genes have been mapped on DNA sequences for commercially related enzyme and product formulations in the medicinal and related industrial fields. Induction and repression are series of enzymatic controls within the cell that regulate metabolism based upon the presence of substrates and metabolic products.

Table II-2

FACTORS CONTRIBUTING TO THE RECALCITRANCE OF CHEMICALS AND POTENTIAL BIOLOGICAL MANIPULATION METHODS

RECALCITRANCE FACTORS		CONTROLLING METHODS*
Biological		
1. Non-existence of an active organism	a,b	Both mutation and selection and plasmid evolution have demonstrated ability for natural as well as artificial systems to recruit genetic codes for catalyzing the degradation of specific chemicals by microorganisms.
2. Absence of comparative biochemistry	a,b	Microbial systems deficient in particular enzymes can either naturally evolve them through the transmission of plasmids or be genetically engineered to have the desired activity inserted as part of their metabolic potential.
3. Lack of enzyme specificity	a	Competitive, non-competitive, irreversible, and allosteric inhibitions can account for the lack of enzyme activity due to the presence of mixed substrates, and repressible metabolites. Genetic engineering can enhance the production of specific enzymes to maintain their presence or evolve new conformational structures.
4. Lack of induction of a requisite enzyme	a	Mutations can be induced in operator, promoter or regulator sequence of DNA enhancing enzyme production (promoter optimization, derepression).
5. Lack of presence of an active organism	d	Bioaugmentation can be used to add active organisms to systems that are deficient in the culture or in maintaining their presence.

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*(a) Mutation and selection (genetic engineering)
(b) Plasmid evolution
(c) Substrate specific enzyme detoxification
(d) Bioaugmentation
(e) Other

(SOURCE: AmTech Consultants)

Table II-2 (Continued)

FACTORS CONTRIBUTING TO THE RECALCITRANCE OF CHEMICALS AND POTENTIAL BIOLOGICAL MANIPULATION METHODS

	RECALCITRANCE FACTORS		CONTROLLING METHODS*
Chemical			
6.	Lack of sufficient energy or carbon for growth	c,d,e	Methods can be developed to concentrate a substrate presence through process modification. A co-substrate could also be added to provide energy and carbon sources. Bioaugmentation can also provide for organisms and free or immobilized enzymes could be added to provide intimate contact with a substrate.
7.	Lack of an essential nutrient	d	Add nutrients
8.	Toxicity of substrate or metabolic product	a,b,c,e	Natural or artificial mutation could select for more fit organisms and plasmid systems could evolve for detoxifying the material. Enzyme detoxification may also be performed independent of cellular maintenance. Substrate dilution to optimum concentrations could also be applied.
9.	Inhibition or inactivation of extracellular enzyme	a,c,d	Same as (3); immobilized or free enzymes could be used to pretreat inhibitor or to saturate its activity. Bioaugmentation could also be applied for the same purpose.
10.	Low concentrations of a substrate	a,c,d,e	Bacteria could be genetically engineered for tenacious removal of the substrate from solution; same as (6).

(SOURCE: AmTech Consultants)

Table II-2 (Continued)

FACTORS CONTRIBUTING TO THE RECALCITRANCE OF CHEMICALS AND POTENTIAL BIOLOGICAL MANIPULATION METHODS

	RECALCITRANCE FACTORS		CONTROLLING METHODS*
Physical			
11.	Failure of chemical to penetrate the cell	a,c,e	Genetic engineering may be used to enhance production of permeases and hydrolases to depolymerize insoluble materials and promote their cellular transport. Immobilized or free enzymes may also be used to provide for direct contact with a substrate. Chemical could also be emulsified on a carrier or pretreated to more readily assimilated forms.
12.	Inaccessibility of substrate	a,c,e	Same as (11)
13.	Complexity of chemical substrate	a,c,e	Same as (11)
14.	Inaccessibility of functional group on substrate	a,c,e	Same as (11)
15.	Environmental factor exceeding microbial tolerance	e	The factor should be defined and engineering adjustments made to adjust the level within tolerable limits

(SOURCE: AmTech Consultants)

In some cases induction in an enzymatic pathway can be regulated by metabolites. For example, Connors and Bornsley (1980) have shown that salicylate or salicylaldehyde is responsible for inducing the prior enzyme sequences necessary for the degradation of naphthalene.

Enzymes can also be affected through non-genetic processes that inhibit particular reaction rates. These can be considered non-genetic and are: competitive; non-competitive; and allosteric inhibition. Competitive inhibition occurs when a differing substrate is structurally similar and reversibly complexes the enzyme. Non-competitive inhibition is an irreversible binding of the enzyme to an unnatural substrate. Allosteric inhibition is, for example, feed-back inhibition and affects the normal metabolic process of cellular metabolism.

Mutations in the operator, promoter or regulatory regions of the genetic codes for the enzymes can serve to increase the probability of RNA polymerase binding or derepress the regulator genes allowing for enhanced enzyme productions in new cell variants. In addition mutations in the structural code of the enzyme can provide for new conformational properties of the enzyme (Hopwood, 1981).

The addition of cell lines through bioaugmentation can allow for the presence of microorganisms to degrade a specific waste should they be absent in a system. For example, Chakrabarty was able to demonstrate the efficiency of his artificial organism (AC 1100) in degrading 2,4,5-trichloro phenoxyacetic acid in garden plots (Chakrabarty, 1981b) and a commercial formulation, Hydrobac^{TM}, can grow on 17 toxic organics as a sole source of carbon (McDowell, 1981) (Table II-3).

2. Chemical

Chemical factors imparting recalcitrance in biological systems are associated with the structural characteristics or concentration of the substrate and other chemical factors external to the environment of the cell. For systems that are deficient in carbon and energy for growth, a co-substrate can be added and the pollutant co-metabolized. For example, a carbon source is required for cyanide destruction through biological techniques.

The importance of co-metabolism is related to constitutive and induced enzymes (Omori and Alexander, 1978). A cell line that constitutively produces an enzyme that acts upon the substrate can be added to a system through bioaugmentation and its growth sustained through the addition of co-substrates.

Substrates are not specific to a cell in most natural systems and, if they were, more pure cultures would be readily isolated. Co-metabolism, or the sharing of metabolism between cells, is most frequently observed (Jacobsen et al., 1980).

Table II-3

SELECTED ORGANIC CHEMICALS THAT CAN BE DEGRADED BY BLENDED SPECIALIZED BACTERIAL SEED CULTURES (McDowell, 1981)

Class of Chemical	Chemicals Tested	Extent of Growth (1)
Aliphatic Hydrocarbons	Paraffin Oil	Heavy
Aromatic Hydrocarbons	Biphenyl	Heavy
	Anthracene	Moderate
	Napthalene	Moderate
	Phenanthrene	Moderate
	Acenapthylene	Light
Halogenated Aromatics and Phenols	Phenol	Moderate
	O-chlorophenol	Light
	p-Bromophenol	Moderate
	2, 4-Dichlorophenol	Light
	Pentachlorophenol	Moderate
	2, 2-Dichlorobiphenyl	Heavy
Halogenated Aliphatics	1, 2-Dichloroethane	Heavy
	1, 1, 2 Trichloroethane	Heavy
Cyanides & Nitriles	Sodium Cyanide	Light
	Acrolein	Light
	Acrylonitrile	Heavy

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(1) Visual evaluation of growth on noble agar plates containing minimal salts and the specified chemical at 100 ppm as a sole carbon source.

Using settled domestic wastewater as a natural seed and a yeast extract as co-substrates, Tabak et al. (1981) was able to demonstrate the level of biodegradation of the following compounds at relatively dilute (5-10 ppm) concentrations.

Table II-4

BIODEGRADATION POTENTIAL OF DILUTE TOXIC WASTES (Tabak et al., 1981)

Phenolics	Performance
Phenol	D
2-Chlorophenol	D
2,4 chlorophenol	D
2,4,6 Trichlorophenol	D
Pentachloro phenol	A
2,4 Dimethyl phenol	D
p-Chloro-m-cresol	D
2-Nitro phenol	D
4-Nitro phenol	D
2,4 Dinitro phenol	D
4,6 Dinitro-o-cresol	N
Phthalate esters	
Dimethyl phthalate	D
Diethyl phthalate	D
Di-n-butyl phthalate	D
Bis-(2-ethyl hexyl) ph.	A
Di-n-octyl phthalate	A
Butyl benzyl phthalate	D
Napthalene	D
2-chloro naphthalene	D
Acenapthene	D
Acenapthylene	D
Halogenated Ethers	
Bis-(2-chloroethyl) ether	D
2-Chloroethyl vinyl ether	D
4-Chlorodiphenyl ether	N
4-Bromodiphenyl ether	N
Bis-(2-Chloroethoxy) methane	N
Bis-(2-Chloroisopropyl) ether	D

Aromatics	Performance
Benzene	D
Chlorobenzene	D,A
1,2 Dichlorobenzene	T
1,3 Dichlorobenzene	T
1,4 Dichlorobenzene	T
1,2,4 Trichlorobenzene	T
Hexachlorobenzene	N
Nitrobenzene	D
Ethylbenzene	D,A
Toluene	D
2,4 Dinitrotoluene	T
2,6 Dinitrotoluene	T
Polyaromatics	
Anthracenes	A
Phenanthrene	D
Fluorene	A
Fluoranthene	A,N
1,2 Benzanthracene	N
Pyrene	D,N
Chrysene	A,N
Polychlorinated biphenyls	
PCB-1016	N
PCB-1221	D
PCB-1232	D
PCB-1242	N
PCB-1248	N
PCB-1254	N
PCB-1260	N

D Significant degradation with rapid adaptation
A Significant degradation with gradual adaptation
B Slow to moderate biodegradative activity, with significant volatilization
C Very slow biodegradation with long adaptation period
T Significant degradation with gradual adaptation followed by a deadaptive process (toxicity)
N Not significantly under conditions of test method and/or precluded by volatilization

The importance of concentration on the rate of enzymatic reactions degrading chemicals of environmental concern cannot be overstated. At extremely low concentrations the velocity of the reaction will be reduced as described in Monod Kinetics. This has been observed by Boethling and Alexander (1978) where they have pointed out the problems of extrapolating high concentrations from laboratory or microcosm studies to actual environmental conditions and expressed a concern for a possible threshold below which concentration a reaction does not proceed.

Thus, when there is insufficient carbon or energy for microbial growth or there are low concentrations of a substrate, a co-substrate can be added to a system as well as bioaugmentation and/or extracted enzymes applied. There may, however, be a threshold under which no reaction will take place. This threshold may be reduced by adding free enzymes rather than through bioaugmentation. If concentrations are still below a reaction threshold the chemical may, in fact, not pose an environmental problem. On the other hand, the source of influent could be isolated and a more concentrated stream obtained for treatment.

Increased or high concentrations of chemicals can pose inhibitory or toxic effects. Toxicity has been quantitatively observed for both aerobic (Szetela and Winnicki, 1980) and anaerobic systems (Pearson, et al., 1980) in a manner that follows similar curves of inhibition. Nevertheless, microbes have a propensity to overcome toxicity, either by developing new enzymatic pathways, by enzymatic detoxification of the toxin, or through developing physiological structures more compatible to a harsh environment. Considering the rapidly dividing primitive nature of unicellular organisms in contrast to slow dividing, highly differentiated cells of higher organisms, the capacity for microbes to adapt and evolve are great and consequently are found in many environmental extremes.

For cell systems a concentration plateau of optimum degradative activity exists. Extracted enzymes, however, are not dependent upon the viability of the cell and can detoxify at higher substrate concentrations. There are several chemical or structural factors that impart a general recalcitrance to a substrate and no specific rules have been developed other than the number and type of substituent groups and overall molecular complexity.

The literature is diverse in the area of examples for establishing general rules especially in the case of halogenated compounds. This includes work associated with the effect of chlorine substitution on PCB utilization (Furukawa et al., 1978, 1979), halogen substituent effects on aliphatic acids and alcohols (Dias and Alexander, 1971), for halogenated aromatics (DiGeronimo et al., 1979; Haller, 1978) and terminal branching factors (Shaeffer et al., 1979).

In the catabolism of aliphatic or hydrocarbon chained compounds, microbial utilization usually takes place through beta oxidation to yield a two-carbon unit that enters the tricarboxylic acid cycle.

Substitutions where beta oxidation occurs (α substitution) are most severe (Dias and Alexander, 1971).

Degradation of halogenated aliphatics occur both aerobically and anaerobically; however, Bouwer et al. (1981) have shown a greater efficiency in anaerobic catabolism of 1- and 2-carbon halogenated compounds such as trichloroethylene, a widely used industrial solvent. On the other hand, aromatic compounds are more efficiently degraded aerobically due to the requirement of oxygenases to cleave rings, though anaerobic degradation has been observed for ring compounds such as phenols (Neufeld et al., 1980).

Inorganic substitutions on aromatic structures pose complex effects that are difficult to generalize. The placement and number of the substitutes in the structure of the phenol ring determine the level of resistance to biodegradation. The effect of these substituent positions on recalcitrance have been demonstrated in the phenolic, benzoic and phenoxy herbicides.

3. Physical

The main physical or morphological factors that impart a chemical's resistance to microbial degradation are the material's insolubility, size, and degree of polymerization. Many waste compounds can be high molecular weight or polymeric and cannot pass into the cell. These compounds are utilized by the action of hydrolytic enzymes into subunits which are subsequently transported through the cell wall into the cytoplasm by permease systems. The enzymatic hydrolytic cleavage can occur on cellular surfaces or in surrounding fluids.

Insolubility alone is not a limiting factor to biodegradation but is a function of the surface area of the particles that insoluble materials are sorbed upon. Increasing the solid-liquid interface can promote the degradation of relatively insoluble pollutants (Wodzinski and Larocca, 1977).

On the other hand, enzyme systems, for example peroxidase, can serve to polymerize soluble aromatic compounds to insoluble forms for subsequent separation (Klibanov, 1981).

The following are toxic pollutants that have been reported to concentrate on solids in biological treatment facilities.

Table II- 5

TOXIC POLLUTANTS REPORTED TO CONCENTRATE IN SLUDGE (Convery et al., 1980)

Acenaphthene
1,2 Dichlorobenzene
1,3 Dichlorobenzene
Fluoranthene
Naphthalene
N-nitrosodi-n-propylamine
Bis (2-ethylhexyl) phthalate
Butyl Benzyl Phthalate
Di-n-bytyl Phthalate
Dimethyl Phthalate
Benzo(a) pyrene
Benzo(u) Fluorauthene
Chrysene
Acenaphthylene
Fluorene
Phenanthrene
Pyrene
Tetrachloroethylene
Toluene

Genetic engineering for enhanced production of hydrolases (e.g., cellulases), the use of free or immobilized enzymes, or non-biological means of pretreatment can assist in overcoming some of the physical factors contributing to recalcitrance.

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III. BIOLOGICAL TECHNOLOGIES CURRENTLY AVAILABLE

Biological systems for toxic waste management primarily use microbial populations to destroy the waste by converting it to cell mass or organic/inorganic products. Biological systems can also sorb or concentrate organic and inorganic materials onto cellular tissue and discharge the toxic material in or on dead cell fragments. Biological systems alter the external environment sufficiently to chemically hydrolyze (e.g., sulfur oxidation by *Thiobacillus* to form an acidic environment) or insolubilize heavy metals (e.g., hydrogen sulfide formation by *Desulfovibrio* followed by precipitation to highly insoluble metallic sulfides). When solids are discharged from a waste treatment system, they are termed sludge, together with whatever other insoluble, precipitated, or added material (flocculant) is present. When toxic materials are bound to well stabilized biological sludge, they are slowly released from the dead cell matrix through further microbial decay, e.g., in land farms or landfills, by saprophytic organisms, and the toxics further metabolized. Efficient treatment methods should be able to destroy the majority of toxicity in the primary system.

A. Biotechnology Descriptions

There are numerous biological technologies available to treat hazardous toxic wastes and are divided into six general categories.

- conventional aerobic and anaerobic processes (Metcalf & Eddy, 1972; Mulligan and Fox, 1976)
- land disposal techniques (Brown, 1980; Concawe, 1980)
- enzyme systems (Munnecke, 1977; Daheny, 1980)
- specialized fermentation systems (Peppler, 1979)
- spill cleanup techniques (Walton and Dobbs, 1980)
- hazardous waste remedial cleanup techniques (Shuckrow, 1981)

Table III-1 summarizes the types of technologies within these categories, their applications and cost, limiting factors and environmental risks. In addition to biological technologies, physical/chemical methods may be used to pretreat persistent waste to forms more amenable for biodegradation in conventional waste treatment systems (Worne, 1981).

1. Conventional Biological Treatment

These microbial degradation processes can occur completely within an enclosed and contained reactor having a long solids retention time (recycle or fixed film), in a land farming operation, or in a series combination.

Table III-1

SUMMARY OF BIOTECHNOLOGIES TO TREAT HAZARDOUS WASTE

Biotechnology	Application and Cost	Limiting Factors	Environmental Concerns
1. CONVENTIONAL			
Activated Sludge	Can be applied in many different modes. An aerobic system that treats primarily soluble organics with a concentration up to 4,000 mg/l. Costs vary according to waste, system, and size. Approximate cost $2-3/1,000 gals. Used in paper and pulp mills, refineries, steel, textile, pharmaceutical, chemical and similar industries. Applicable to substituted aromatic compounds as well as simple hydrocarbons.	● Cost of aeration high ● Limited to soluble wastes at low-medium concentration ● Sludge requires stabilization though some systems produce well oxidized sludge	Volatilization of toxics Aerosols Sludge disposal
Aerated Lagoons	Similar to activated sludge, though not as efficient. Destroys soluble organics to 3,000 mg/l but requires greater volume. Used in paper and pulp mills, refineries, petrochemical, and textile plants. Approximate costs are $2.00-2.50/1,000 gals.	● Seasonal variations ● Limited to soluble wastes ● No operational controls ● Low efficiencies ● Requires sludge removal ● Could have anaerobic zones	Volatilization of toxics Aerosols Sludge disposal

(SOURCE: AmTech Consultants)

Table III-1 (Continued)

SUMMARY OF BIOTECHNOLOGIES TO TREAT HAZARDOUS WASTE

Biotechnology	Application and Cost	Limiting Factors	Environmental Concerns
1. CONVENTIONAL (Cont.)			
Trickling Filters	A downflow fixed bed system upon which the organisms grow and operate aerobically. Removal of soluble organics up to 2,000 mg/l. Applicable to substituted aromatics. Primarily used as a preconditioning process. Applicable in same waste streams as activated sludge. Approximate costs are $1.50-2.50/1,000 gals.	• Low efficiencies • Low control of biomass • Limited to soluble wastes	Sludge disposal
Stabilization Ponds	Can be operated aerobically or anaerobically with no aeration. Are low in capital costs and can accept highly concentrated waste. Not applicable for aromatic degradation. Anaerobic sludge could precipitate heavy metals as sulfides. Simple to operate and accepts pulse loadings. Approximate cost $3.50-4.00/1,000 gals.	• Requires long retention • Inefficient use of volume • Requires sludge removal	Volatilization Possible odors
Anaerobic Digestion and Variations (Contact and Fixed Film)	Can be operated in many different modes. Does not require oxygen. Can treat soluble high strength waste and insoluble sludges. Inefficient on aromatics and efficient on non-ringed hydrocarbons. Generates methane gas and can be recovered. Cost approximately $4.50-5.00/1,000 gals without energy credit. Can control volatile off-gases. Applied on paper wastes, food industries, petrochemical, and municipal sewage. High temperature, thermophilic, systems may be applicable to some wastes.	• Requires long retention • Inefficient on highly aromatic compounds • Inappropriate for dilute wastes • Prone to upsets • Require careful controls	Methane Pressures

(SOURCE: AmTech Consultants)

Table III-1 (Continued)

SUMMARY OF BIOTECHNOLOGIES TO TREAT HAZARDOUS WASTE

Biotechnology	Applications and Cost	Limiting Factors	Environmental Concerns
2. LAND DISPOSAL			
Land Farming	Aerobic process where sludge primarily from waste treatment processes are ultimately disposed on land, sludge is harrowed into soil frequency depending on loading. Commercial fertilizer added. Runoff controls. Applications to 200 tons solid per acre/yr. Overall costs $12-18 ton wet weight. Used primarily by refineries but also limited use with leather tannery and petrochemical wastes.	● Cost of transport ● Biodegradable materials ● Seasonal fluctuations ● Soil characteristics	Volatilization Leaching and runoff Food chain crops Reclamation
Composting	Ground relatively dry waste is piled in windrows or spread evenly over land. Similar to land farming. Mechanical systems such as rotating drums can also be used. Cost approximately $20-30/ton dry weight.	● Cost of transport ● Biodegradable materials ● Seasonal fluctuations	Volatilization Leaching Food chain crops Dust suspension

(SOURCE: AmTech Consultants)

Table III-1 (Continued)

SUMMARY OF BIOTECHNOLOGIES TO TREAT HAZARDOUS WASTE

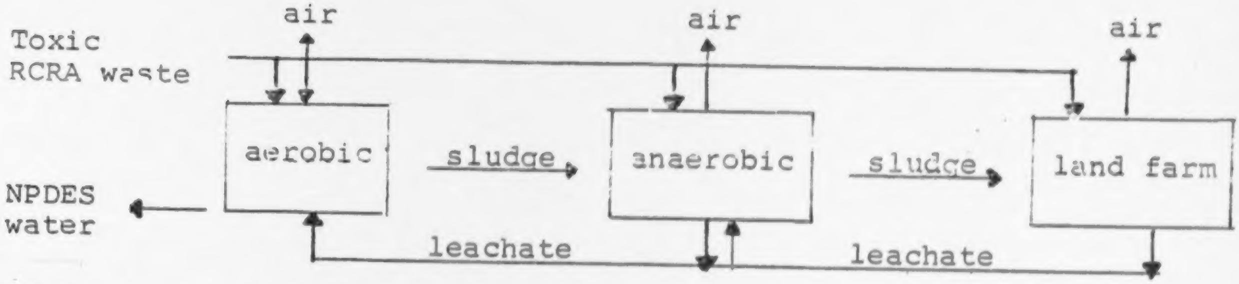
Biotechnology	Applications and Cost	Limiting Factors	Environmental Concerns
3. ENZYME DETOXIFICATION			
Free or Immobilized Enzyme Systems	Highly selective applications. No commercial use known to date though trials conducted for broad specificity applications to organophosphate pesticides. Has also been demonstrated in converting phenols. Expensive to extract enzymes though genetic engineering may allow for less expensive forms. Could be used in detoxification of commercial formulations. Widely used in food industry. Peroxidase system has been shown to polymerize multiple types of soluble aromatics.	• Cost of enzyme extraction • Specificity to substrates • One or two reactions (non mineralization)	Production Handling
4. SPECIALIZED FERMENTATIONS			
Immobilized or Free Cells	Highly controlled and mixed fermentation systems used primarily in the pharmaceutical industry for production of antibiotics and other medicinals. May have applicability in treatment of concentrated hazardous waste. Cost estimates are unknown. Systems would be applicable to improved cell lines.	• Cost of mixing • Cost of cells	

(SOURCE: AmTech Consultants)

SUMMARY OF BIOTECHNOLOGIES TO TREAT HAZARDOUS WASTE

Biotechnology	Applications and Cost	Limiting Factors	Environmental Concerns
5. SPILL CLEANUP SYSTEMS	A variety of biological techniques can be used in response to spills and are applicable when (1) material is biodegradable, (2) a formulation is available and (3) no means are available or contamination does not require rapid removal. Costs could range from $5,000-100,000 per incident or greater. Mobile reactors, surface impoundments, surface or ground water bioaugmentations are applied. Have been used in oil, orthochlorophenol, and acrylonitrile spills.	● Length of time available ● Hazard of waste ● Containment structures ● Temperature effects ● Oxygen and nutrients ● Quantity and quality of spill ● Availability of cell line ● Site geology	Aquifer contamination from forced leaching Volatilization
6. REMEDIAL ACTIONS	Similar to spills but of an unknown quantity, quality and extent of contamination. Are biological applications at abandoned hazardous waste sites or on-site systems requiring upgrading. These activities can range from installation of any conventional technology, aeration of impoundments, in situ bioaugmentations, or enhancement of native microorganisms. Costs are unknown but could approximate $100,000 per year per surface impoundment retrofitted.	● Site geology ● Temperature effects ● Oxygen and nutrients ● Characterization and biodegradability of waste	Time for aquifer decontamination Volatilization

(SOURCE: AmTech Consultants)



(SOURCE: AmTech Consultants)

Aerobic systems can oxidize about 50 percent of the waste organic matter into carbon dioxide and water and convert the remaining organics to cell mass (sludge). Some intact waste is also discharged in the effluent or volatilized. The cell yield, mass of cell/mass of organic, is about 40-60 percent for aerobic systems. Anaerobic systems are less efficient in the production of biomass, with a cell yield of 15-25 percent, but convert a majority of the incoming waste to methane and carbon dioxide gases which are vented or incinerated (flamed). Thus, in a properly designed aerobic/ anaerobic system operating in series, about 10-15 percent of the incoming organic matter ends up as viable anaerobic cells and cell fragments for ultimate disposal. The process is frequently termed stabilization. A high percentage of this sludge is inorganic, making the total sludge requiring disposal 20-25 percent of the inorganic and organic solids entering a system (ash and volatiles). By scavenging their own cells, aerobic systems with long solids retention times can reduce the amount of sludge produced.

If toxic organic wastes are readily biodegradable, they will not accumulate in the sludge and will be metabolized into cellular material and inorganic endproducts as normal substrates. However, if they are recalcitrant and present with more readily degradable wastes, the growth kinetics are less than for the microbial population on the whole. Thus, the system could be underdesigned for the particular toxic substrate and degrading microbe in question. In this case, degradation will not follow the normal stabilization pattern and accumulation of the toxic in the effluent water or sludge will occur. Also, if the solids retention time is less than a particular population's reproductive period, washout will occur and productive populations will not establish within the system. Therefore, it is important to know microbial growth kinetics for particular toxic wastes (substrates) as well as for their degrading microbes, and system design or bioaugmentation practiced to effect as complete toxics destruction as possible.

Reactor Types

The major reactor configurations used in biochemical operations are: 1) the continuous stirred tank reactor; 2) the batch reactor; 3) the plug flow reactor; 4) the packed tower; and 5) the rotating disc reactor.

A continuous stirred tank reactor often has a liquid stream containing a substrate entering and a stream of microbial slurry leaving. Mixing is sufficient to make the concentration uniform throughout.

A batch reactor has no flow in or out, but rather a batch of material is placed into a vessel, innoculated, and growth is allowed to occur. As growth progresses the reaction conditions change and consequently so does the growth environment.

The plug flow reactor may be considered as a moving batch culture because each increment of flow moves through the reactor unaffected by the increments on either side. Microorganisms at different points in the reactor will be in different physiological states. Recycle is needed to innoculate the reactor so substrate removal can occur.

A packed tower is a fixed film reactor where the organisms grow as a film on an immobile substance such as rock or plastic media (immobilized cells). The fluid runs over the medium in a thin film as opposed to being submerged.

A rotating disc reactor has microorganisms growing as a film on discs which are rotated in the liquid. When recycle is added the reactor acts as an intermediate between a slurry reactor and a fixed film reactor.

a. Types of Aerobic Treatments

Activated sludge. The activated sludge process consists of a reactor and a settler. In the reactor a flocculent slurry of microorganisms is used to biodegrade soluble organic matter. Insoluble organic and inorganic matter are also treated in the reactor mainly by being adsorbed or entrapped within the microbial floc particles. A settler is used to separate the organisms from the treated wastewater prior to discharge and is also used to recycle a concentrated slurry of microorganism from the settler under flow to the reactor to maintain a desired cell concentration within the reactor.

The primary objective of activated sludge is to biodegrade soluble organic matter although insoluble matter will be entrapped within the microbial floc and treated as well. The activated sludge process is usually used with wastewater in which the soluble T_bOD (Total Biochemical Oxygen Demand) lies between 50 and 4,000 mg/l.

There are seven major types of activated sludge: conventional (CAS), tapered aeration (TAAS), step aeration (SAAS), contact stabilization (CSAS), completely mixed (CMAS), extended aeration (EAAS), and pure oxygen (POAS).

Conventional activated sludge is the most frequently used type of process and is found at numerous industries discharging toxic waste in low concentrations. These include petrochemical and chemical plants, petroleum refineries, and leather tanning industries. Aromatic compounds are readily degraded through aerobic processes and activated sludge systems have demonstrated over 95 percent destruction of toxic compounds such as phenols, nitrophenols, trichloroethylene, and chlorinated benzenes. Systems such as contact stabilization activated sludge and pure oxygen activated sludge, the latter where high speed submerged turbines are employed, have the potential of biodegrading some of the more recalcitrant wastes; however, energy requirements could be cost limiting. Costs for activated sludge systems can range from $2-3 per 1,000 gallons and from $0.1 to $5 per pound organic destroyed. These values vary depending upon oxygen demand of the waste.

Aerated Lagoons. Completely mixed aerated lagoons are generally used for two purposes. First, they are used to convert soluble organic matter into microbial cells as a pretreatment prior to sedimentation. Second, they can serve to oxidize organic matter as the primary means of waste treatment. They are generally earthen basins 5-20 feet deep lined with clay or plastic. Mixing energy supplied must be large enough to keep all solids in uniform suspension and provide an adequate supply of oxygen. Aeration and mixing is usually provided by mechanical surface aerators. The biological population is usually quite complex.

The merits of aerobic lagoons are that they are easy to operate, are relatively resistant to upsets and pH shock loads, and can treat wastes with high temperatures without the requirement for additional cooling.

The main defects are that microbial kinetic parameters will vary with temperature limiting the degree of treatment with season, and that there are few operational controls over the system parameters.

There are many surface impoundments or lagoons which could be upgraded at industrial sites to aerobic lagoons for the treatment of toxic wastes. Aerobic lagoons can handle the same type of waste as activated sludge treatment systems and are slightly less expensive per volume throughput. However, they are not as efficient on a cost per mass destroyed basis as activated sludge.

Aerobic Digestion. The main objective of aerobic digestion is the destruction of insoluble organic matter. Aerobic digestion is more applicable to microbial sludges generated by primary biological processes. Raw sludge may contain high soluble substrates, thereby exerting a large oxygen requirement. Aerobic digestors can compete with anaerobic digestors in stabilizing primary biological sludge.

Aerobic digesters are usually uncovered concrete tanks about 15 feet deep similar to activated sludge reactors. A complex microbial community exists in the reactor. Mixing and oxygen transfer are accomplished by both bubble and mechanical diffusion devices.

The main merits associated with aerobic digestion include an odorless, biologically stable end product; less operating skills than anaerobic digestion; capital costs are low; and the system can achieve organic solids stabilization of 45-50 percent.

The defects of aerobic digestion are that energy must be expended to meet oxygen requirements; it is highly dependent upon temperature; there is no energy recovery in the form of methane; and aerobic digestion results in more residual sludge than anaerobic digestion.

There are no known applications of aerobic digestors in treating toxic wastes but they may be applicable to toxic aromatic sludges.

Trickling Filters. Trickling filters destroy soluble organic matter through aerobic conversion into microbial cells on fixed media. A trickling filter's major components are: 1) the filter media; 2) an enclosure; 3) a distribution system; and 4) an underdrain system.

The filter media provides the surface on which the microorganisms grow and should allow liquid to flow evenly in a thin film. The media should also be chemically and mechanically stable, and have sufficient void space for the flow of air and to allow the slough of microorganisms to be carried away. The enclosures are usually made of reinforced concrete capable of vertical support if stone media are used. If plastic media are used, the enclosure needs less vertical support. A fixed or rotary distributor system is used to provide a uniform hydraulic load. The underdrain system must do three things. First, it supports the media, second, it provides an uninterrupted path of flow for the liquid to drain, and third, it must allow access of air to the bottom of the filter.

Trickling filters are simple, easy to operate, and tolerate changes in the influent; however, there is no effective way to control the amount of biomass present in the system. They are used in many industries and have been reported to treat the same spectrum of toxic waste that aerobic lagoons and activated sludge systems handle. Their cost is approximately the same as for aerobic lagoons.

Rotating Biological Contactors. A rotating biological contactor (RBC) consists of parallel circular discs attached perpendicularly to a horizontal shaft which passes through their centers. The entire assembly is placed into a tank so that the discs are approximately half immersed. Microorganisms grow on the surfaces of the discs and rotation of the shaft brings the organisms into contact with the liquid allowing the organisms to adsorb the organic matter in the liquid.

As a rule RBCs have a disc diameter of 12 feet and maximum length of 25 feet. Because of the nature of the kinetics of substrate removal, most RBC systems are designed in stages with the shaft mounted perpendicular to the direction of flow.

These systems have been used in Europe for several decades and are found in over several hundred industries in the U.S. They are considered highly practical and find applications in municipalities, and for petroleum and petrochemical wastes.

b. Types of Anaerobic Treatments

Anaerobic Digestion. The primary role of anaerobic digestion has been to stabilize organic solids (sludges) in municipal and industrial wastewater treatment. Anaerobic digestion is well suited to the stabilization of sludges because the large amount of nonmicrobial organic matter present can be reduced substantially with a minimal production of biomass.

Anaerobic digestion is carried out in covered tanks that are either mixed or unmixed. Unmixed reactors are known as standard rate digesters and have long detention times. Mixed reactors are known as high rate digesters and have shorter detention times. Temperatures can be in the mesophilic or thermophilic range, the latter at much faster reaction rates. The Chicago Metropolitan Sanitary District is converting all of their digesters to thermophilic.

Anaerobic digestion is carried out in three stages by three bacterial processes. The first process, known as liquefaction, is a hydrolysis of insoluble material to soluble complex organics. The second stage, acidogenesis, converts complex organic compounds into simple organic acids. The third group of organisms, known as methanogenic bacteria, convert the organic acids to methane and carbon dioxide. The methanogens are rate limiting because they are extremely sensitive to changes in pH requiring a condition 6.8-7.6, and are extremely slow reproducers (3-5 days). Thus the rapidly metabolizing acidogens, if overfed, can upset the pH of the system by increasing the pool of organic acids. Therefore careful monitoring and control are required.

Anaerobic Contact. Anaerobic contact is an effective means of degrading high strength waste with a soluble T_bOD in excess of 4,000 mg/1. Generally, anaerobic contact is used as a pretreatment technique prior to an aerobic operation because the anaerobic effluent will contain too much residual organic matter for direct discharge. This process has found application to waste waters from the meat-packing, brewing, pharmaceutical, chemical and food processing industries. In the contact process the resulting biomass is separated from the effluent and a portion recycled to the reactor to maintain a high cell to food ratio. Hydraulic retention times have been reported at less than a day.

Anaerobic Fixed-Film Treatment Processes. The main anaerobic fixed-film treatment process is the anaerobic filter. The anaerobic filter is used for the treatment of both domestic and industrial wastes. The anaerobic filter is a column filled with some solid medium used for treating the organic matter in waste water. The waste flows upward through the column and the organic matter is contacted by the bacteria growing on the media. Celanese Corporation has performed extensive research on an anaerobic filter system that treats effluent from its petrochemical plants, such as acetic and formic acid, acetaldehyde, and acrylic acid at reported influent levels up to 15 percent. Considering the sale of the methane gas the system can pay for itself in 4-5 years depending on the waste.

Costs for anaerobic systems are greater than aerobic on a volume basis but are an order of magnitude less on a per mass destroyed basis.

All of the above conventional systems can degrade toxic organic waste, the efficiency dependent upon the kinetics of the particular organism to substrate, and type of system used. Because toxic materials are frequently found in the sludge from industry discharging and treating them, increasing process efficiency would decrease the concentrations of particular toxics in the waste. Several of the above systems might be applicable as on-site treatment facilities for toxic sludge: primarily the aerobic and anaerobic digestors and stabilization ponds. Generally, aromatic compounds are more readily treated aerobically. For chemical organic wastes that are in the dissolved form, extended aeration activated sludge would serve to reduce the total quantity of sludge discharged and provide the increased solid retention for organic recalcitrant wastes. For off-site hazardous waste treatment, leachate streams could be treated by any number of the above aerobic processes. Toxic liquid waste could also be diluted and treated accordingly by aerobic processes.

2. Land Disposal

Land Farming

The biological decomposition of organic compounds can be accomplished through applications to soil in a process commonly referred to as land farming or land application. Land farming implies that a crop of microorganisms are being grown utilizing the waste as a carbon and energy source, though plant crops are also grown. Several texts have recently been published on the practice (Overcash, 1979, 1982) and the Environmental Protection Agency includes land farming as a disposal method in its hazardous waste regulations (Brown, 1980). There are over 150 land farming operations in the United States ranging up to 1,500 acres in size. The petroleum refining industry uses the majority of these sites for the disposal of refinery wastes, such as API separator sludge. They are predominantly located in southern states (Rucker, 1981).

Land applying toxic materials is a highly controversial subject (BioCycle, 1981) with some practitioners advocating that nearly all toxic organics can be broken down safely in land systems (Overcash, 1981) whereas others take a cautionary approach in addressing the subject (Suntech, 1981).

A number of factors influence the rate at which organic compounds are degraded in soils: 1) nature of the compound; 2) soil aeration; 3) soil pH; 4) soil temperatures; 5) soil moisture content; 6) nutrient status of the soil; 7) the size and density of the soil microbial population; and 8) the presence of substances toxic to microorganisms. Each of the factors is important in affecting the dynamics of the decomposition of organics in soil and should be considered in designing systems using soil for waste disposal.

Organic compounds are normally degraded most rapidly under aerobic soil conditions when soil pH values are near 7. Anaerobic degradation of organic compounds in soil is normally slow, but a few types of materials (e.g., DDT) are decomposed more rapidly under anaerobic conditions than in aerobic environments. Under acid conditions fungi will be the predominant type of microorganisms. At near neutral pH values balanced microbial population will be present in soil, whereas at high pH only bacteria will be present.

The rate of decomposition of organic compounds in soil increases with increasing temperature from slightly over $0^{\circ}C$ to $37^{\circ}C$ and are usually at a maximum when soil moisture tension is maintained at -0.3 to -1 bar. Low temperatures can be a major factor influencing organic loadings of soil during the winter months and, under waterlogged and near waterlogged conditions, bacteria are largely responsible for decomposition.

The nutrient status of the soil is an important factor in determining decomposition rates of some organic compounds. Some of the required nutrients may be present in the compounds added to soils whereas with other compounds (e.g., hydrocarbons) no N, P, or S is present in the organic materials. In addition, all other elements essential for continued microbial growth (e.g., Ca, Mg, K, Fe, Zn) must be present in soil if rapid degradation of organic materials is to proceed. Inorganic fertilizers may have to be added to some nutrient deficient soils to stimulate decomposition.

It is important that a diverse and abundant population of microorganisms be present in soil if a variety of organic materials is to be rapidly decomposed. The addition of a decomposable substitute to soil usually results in a marked increase in the population of microorganisms (a few species) capable of obtaining energy from degradation of the compound. An initial lag in degradation of the compound is initially observed and the population will expand rapidly because of the competitive edge given the microorganisms capable of obtaining energy from the added compound.

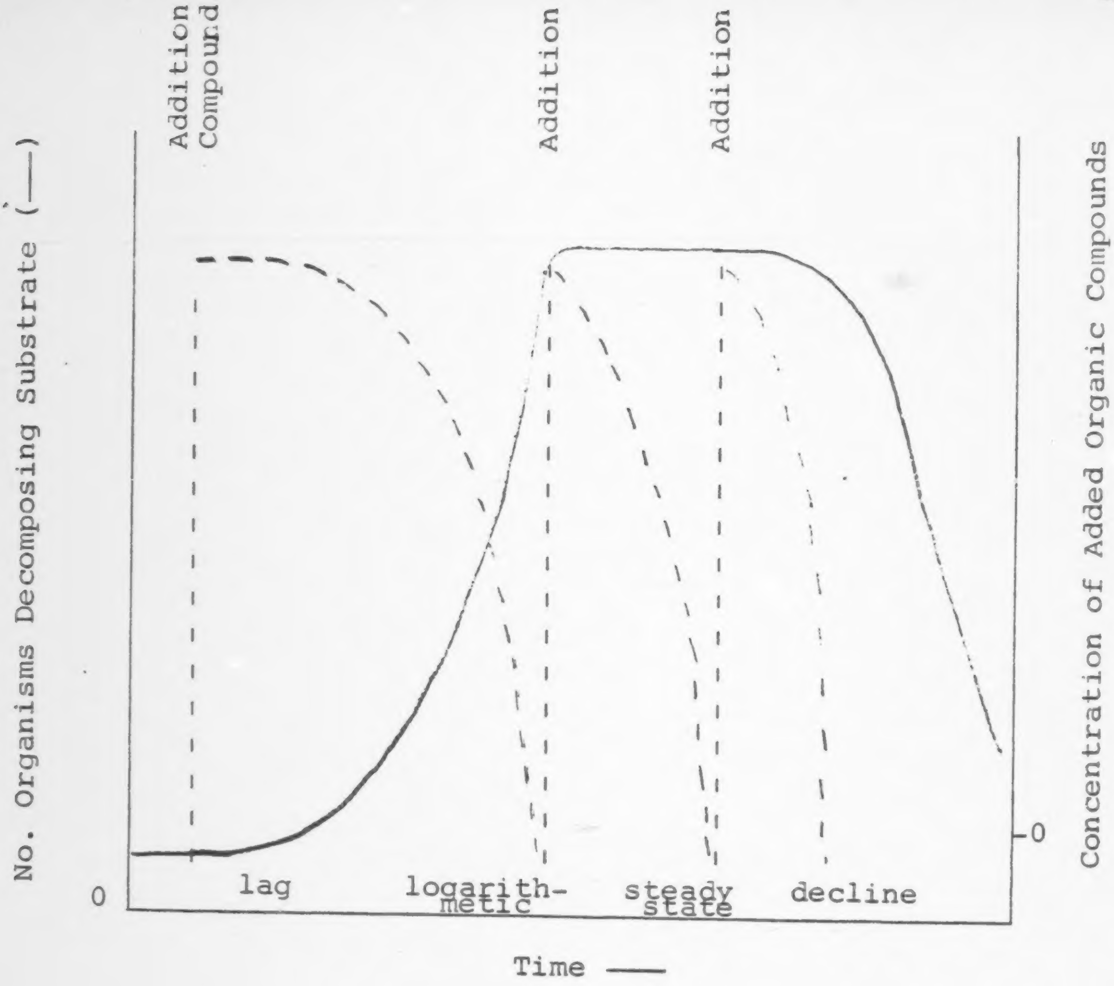


Figure III-1

HYPOTHETICAL RESPONSE OF THE MICROBIAL POPULATION FOLLOWING MULTIPLE APPLICATIONS OF AN ORGANIC CHEMICAL TO SOILS (Nelson, 1981)

When an organic material is added to a soil containing high levels of microorganisms capable of degrading the compound, no lag phase will be observed (above). The population size will tend to decline to near original values when the compound is no longer added to soil.

Addition of inorganic or organic substances that are toxic to the hetertrophic organisms (decomposers) in soil may severely reduce the rate of biodegradation. Heavy applications of certain pesticides, heavy metals, phenols, cyanide, etc., may significantly reduce the soil microbial population or inhibit decomposition of organic compounds.

If properly managed the soil is an effective biological degrader that has the capability of decomposing most types of organic or toxic substances and poor soil management procedures will lead to conditions where degradation of organic compounds is slow and where offensive products (e.g., mercaptons, sulfides, volatile acids) are formed. The difference between successful and unsuccessful land disposal systems for organic materials lies in proper design and management of the site.

Two types of waste disposal operations fall under the category of land farming: 1) decomposition without nutrient recovery and 2) decomposition with nutrient recovery by plants (recycling wastes on cropland). In the first case the objective is to dispose of large amounts of waste on limited land areas (maximum loading rate) with no attempt to recover degradation products. In the second case wastes are used as organic fertilizers to produce marketable food, feed, or fiber. The amount of waste applied is that which meets the nutrient needs of the crop and, thus, a balance between nutrient additions and removals is achieved.

Only biodegradable wastes are appropriate for land farming. A determination of biodegradability can be made through laboratory or field decomposition tests or through examination of published literature.

Environmental problems associated with land farming operations include: (1) runoff of organic compounds from treated areas into surface waters, (2) leaching of organic chemicals or metabolites into ground water, (3) leaching of nitrate produced by decomposition of nitrogenous wastes, (4) destruction of soil structure and tilth through compaction, addition of sodium or other salts, or excessive applications of oily wastes, (5) volatilization of organic chemicals during or after application to soils, (6) excessive accumulation of heavy metals or toxic compounds in soil with the possibility of food-chain contamination, and (7) accumulation or leaching of pathogenic organisms present in wastes. The design and operation of a land farming system must be directed at eliminating or minimizing these environmental effects.

Wastes most suitable for application to fallow soils include papermill residues, oil and grease, solvents, starch, cellulose, etc. Waste loading rates are normally determined by either the hydraulic loading permitted by the soil (dilute wastes) or the organic assimilative capacity of the soil. Other considerations (e.g., toxic metals, salts) may affect the loading rate of certain wastes. Rates are normally calculated as kg/ha/year although the actual loading is normally accomplished in several increments throughout the year. In some cases maximum loading rates are determined by cadmium additions to the soil (2 kg Cd/ha/year is the EPA limit for soils supporting food chain crops).

Site management considerations include: (1) proper incorporation of waste into soil, (2) avoidance of soil compaction, (3) maintenance of soil pH, (4) appropriate supplemental fertilization to insure good crop growth, (5) proper seeding, care, and (6) appropriate timing of waste applications in relation to crop growth cycles. Surface runoff from the site should be limited and the soils should be tile drained if required for successful crop production. Soils composition should be monitored yearly to determine if accumulations of waste materials or degradation products is occurring. Crop composition should be monitored periodically to insure that hazardous substances are not being introduced into the food chain.

Characteristics desirable for a land farming disposal system include a level site with moderately permeable, medium-textured soils (e.g., loam, silt loam). Coarse-textured soils (sands) do not possess the adsorptive capacity for retention of organic and inorganic compounds and crop yields on sandy soils are normally low due to poor water holding capacity. Clay soils have large sorptive capacities, but the hydraulic loading rates are low and trafficability is poor during wet periods of the year.

Soils should have at least moderate levels (3-5 percent) of organic matter to provide adsorptive surfaces for hydrophobic compounds. The microbial activity in the soil should be high to promote decomposition of added organic materials. The site should not be in the flood plain of rivers or creeks and there should be at least 90 cm of soil above the seasonal high water table level, sand layers, or fractured bedrock. The disposal site should be remote from dwellings, public buildings, etc., and should be at least 300 feet from public roads.

Sewage sludges, wastes, explosive manufacturing waste, and tannery wastes are examples of material that have been successfully applied to cropped soils. Costs for land farming sludge can be as high as $30 per ton but vary according to the hauling distance and cost of land. Approximate values are $12 - $18 per ton wet weight.

Composting is very similar to land farming but does not necessarily occur in the soil. Composting basically involves grinding or particalizing well oxidized organic material such as sludge to a uniform size and allowing saprophytic organisms to decompose the waste over a period of several months. Toxic waste can be blended with the compost in a form of co-disposal (that is, blending non-toxic and toxic waste). Compost is either spread or piled in windrows and aerated with periodic turning on tilling. An active population of aerobic organisms grow on the compost and can co-metabolize or specifically utilize the toxic substrates. Proper nutrient balance, moisture, pH, and temperatures are required. Composting can be used to produce soil conditioners, e.g., humus, but should be carefully assayed for the presence of residual concentrations of toxic materials. Compost can also be added to contaminated soil to promote decomposition. It is currently being used to reclaim a wood treating facility contaminated with creosote, phenols, and pentachlorophenol.

3. Enzyme Detoxification

Enzyme detoxification is a one- or multi-step sequential reaction that converts a toxic material in aqueous solution into either non-toxic forms or forms that are more readily assimilated by conventional waste treatment systems. Enzyme detoxification can be conducted by free or immobilized enzymes that are extracted from selected cell lines, or can have cells that continuously produce specific enzymes immobilized and embedded in columns or mixed in reactors. Enzymes are usually immobilized on glass beads or other inert solid media providing a high surface exposure in a column or tank, or as freely applied. Applying cells freely that produce specific enzymes is similar to bioaugmentation and using them immobilized in columns or tanks is similar to some conventional treatment technologies. The objective, however, is to perform specific detoxifications rather than total destruction to inorganic end products. Extracted enzymes with broad substrate specificity are sought and usually a hydrolase is involved. For example, parhydrolase extracted from a mixed cell culture of _Pseudomonods_, _Brevi bacterium_, _Azotomones_, and _Xanthomonas_ is able to detoxify eleven commercial organophosphate pesticides to p-amino phenol and phosphoric acid derivatives; phenolase extracted from yeast has been shown to lead to production of CO_2 and water from phenols; and peroxidase from horseradish has been shown to polymerize and precipitate soluble aromatics. Because it is necessary to extract enzymes and maintain their stability in a free or immobilized state, costs have precluded their use in toxic waste treatment. However, with increasing costs of hazardous waste disposal as well as increased efficiency in producing enzymes through genetic engineering or the synthetic gene machines, the cost of enzymes may be significantly reduced and applications may become more practical.

Potential applications include highly specific and isolated waste streams such as in the pesticides and petrochemical industry. Diverse uses could also be found in cleaning containers or tank washings. Costs are difficult to estimate and depend upon the cost of producing the enzyme, the concentration of the waste, activity and decay rate of the enzyme, and the reactors selected. Using minimal enzyme costs a hydrolysis system was costed at less than $1 per 1,000 gallons. Concentration of waste and the cost of enzymes have a major influence on the system.

4. Specialized Fermentations

Specialized fermentations are used in the pharmaceutical and food product industries and are highly controlled well mixed systems operated in batch or continuous flow. They are used to produce antibiotics, vitamins, amino acids and many other secondary metabolites or whole cells using simple or specific raw materials (e.g., molasses) and nutrients as a feed. The systems employ free or immobilized cells and sometimes enzymes. A major concern in the use of these technologies is the need to maintain aseptic sterile conditions to preserve the pure culture required for the reaction.

In hazardous waste degradation these technologies could be adapted for use with specific cell lines. Feed costs would include addition of nutrients and buffers and the reactor would have to be highly mixed and controlled and air supplied depending on whether air is a reactant. Microorganisms may also have to be added to the system. Because the cell line would be highly adapted to the toxic waste it is doubtful that other organisms would survive and contaminate the system but precautions should be taken to assure axenic cultures or maintenance of the mixed culture. Conventional treatment technologies are scaled up slowly mixed non-septic versions, treating diverse mixtures of waste with uncontrolled cultures. A specialized fermentation system would be smaller scale systems, use highly specific waste streams, be closely monitored and controlled, and maintain a pure or mixed culture.

No applications of these technologies are known for hazardous wastes nor cost estimates available.

5. Spill Cleanup Systems

Biological technologies can be used to clean up toxic commercial materials that contaminate either land or water. Some degradable spilled materials are flushed into sewage systems. The principles involved in decomposition of organic materials applied to soils as a disposal operation also apply to accidents involving spillage of organic compounds on land. The major difference is that very high loading rates are often associated with spills, but the affected area is normally small in size. Renovation of soil affected by organic materials should follow several steps: (1) identification of compound or compounds spilled on soil, (2) determination if compounds are biodegradable, (3) amendment of soil with fertilizers, limestone, or mutant strains of bacteria such that decomposition will proceed at maximum rate, (4) uniform mixing of compound with soil in the affected area so that concentrations will be reduced by dilution, (5) monitoring of soil to determine if compounds are degrading.

Because of the high concentrations resulting from spills, surface runoff or leaching into groundwater aquifers occurs. Surface impoundments can be constructed to collect runoff or leachates and treat them biologically through aeration, nutrient additions, and bioaugmentation. Ground waters can also be decontaminated through a variety of methods. Bore holes can be drilled over the site of contamination and leachate withdrawn downgradient from the spill zone. Leachate can then be treated in surface impoundments, mobile reactors, or through spray irrigation over the spill zone to promote further recirculation of nutrients and aerated water. In the latter practice a recirculated stream from the aquifer serves as the fluid for a pseudo in situ trickling filter (Walton and Dobbs, 1980). Contamination of deep aquifers may also be treated in situ. One company has developed a technique to add nutrients and oxygen to promote biodegradation in deep aquifers and groundwaters (Suntech Group, 1981).

Generally, applications can be performed on all biologically degradable compounds but toxicity and public health risks are of first priority in spill situations and, if a significant hazard exists, material will be physically removed in the shortest timeframe possible. If compounds are found to be non-biodegradable or an eminent hazard exists, the soil in the affected area must be transported to a secure landfill or treatment facility or treated on-site through physical/chemical methods.

Applications have been used for petrochemical and petroleum products spilled on- and off-site with documented cases for gasoline, acrylonitrile, and ortho chlorophenol. Costs are site-specific and could range from $5,000-$100,000 or greater per incident but are less than for physical/chemical treatment alternatives, e.g., incineration of contaminated soils.

B. Biotechnology Costs and Comparisons to Alternatives

The cost of hazardous waste treatment is based upon capital, financing, operating, and maintenance costs, plus a fee for services. These values are usually derived by aggregating them on an annualized basis over the useful life of the technology and dividing by the annual volume of the waste treated and reported as $/volume. Discounted cash flows are also frequently used to indicate the payback period on the capital and start up costs, and to estimate the return on investment. Economies of scale generally apply, but exceptions occur. Direct comparisons between costs for differing technologies are difficult as technologies may be restricted to a particular form or type of waste. In addition, more than one type of technology is used in a process train; for example, equalization or pretreatment followed by biological treatment, sludge separation, and sludge disposal.

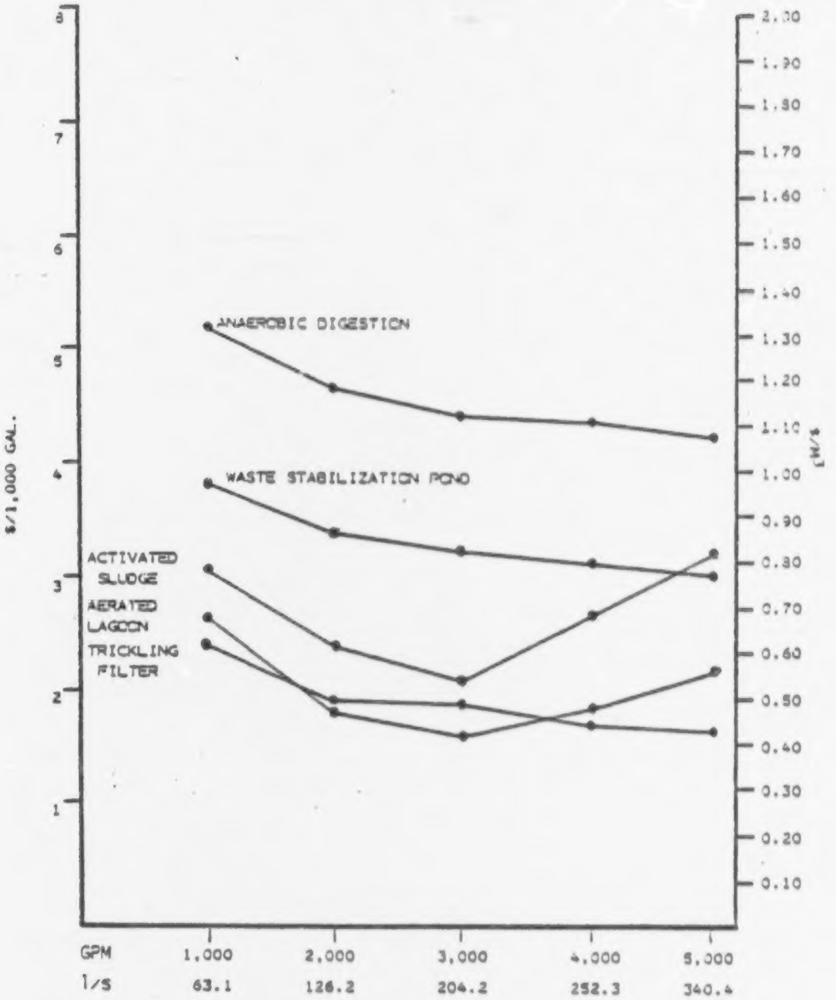
A specific cost analysis is usually required for each individual process/system before making a decision on a waste treatment technology. For example, in a comprehensive analysis of the costs for treating dilute and concentrated phenolic wastes (Blum, 1980), the Air Force considered up to 12 different treatment alternatives. The results, Table III-2, indicate that biological treatments were most cost-effective for very dilute and mildly concentrated phenolic waste streams.

Table III- 2

SUMMARY OF COSTS FOR ALTERNATIVE PHENOLIC WASTEWATER TREATMENT SYSTEMS (BLUM, 1980)

System/Process	Capital Investment	Annual O&M Cost	Total Present Value Cost (TPVC)
I. Aircraft Paint Stripping Waste Water: 10 gpm-2600 mg/l Phenol			
a. Biological	$200,000	$ 30,000	$ 384,000
b. Activated Carbon	430,000	97,600	1,030,000
c. Ozone	980,000	94,000	1,560,000
d. Recovery	500,000	177,000	1,600,000
e. Chlorine Dioxide	142,000	545,000	3,490,000
f. Hydrogen Peroxide	115,000	736,000	4,640,000
g. Potassium Permanganate	135,000	1,613,000	10,000,000
II. Industrial Waste Stream: 1 mgd (694 gpm) - 10 mg/l Phenol			
a. Biological	282,000	101,000	903,000
b. Ozone	670,000	54,000	1,000,000
c. Chlorine Dioxide	92,000	219,000	1,440,000
d. Activated Carbon	940,000	95,000	1,500,000
e. Hydrogen Peroxide	50,000	315,000	2,000,000

Figure III-2 presents the comparison of life-cycle average costs for conventional waste treatment technologies, based upon economies of scale (SCS, 1980).



GPM-gallons per minute; l/s-liters per second

Figure III-2

COMPARISON OF LIFE CYCLE COSTS
FOR CONVENTIONAL BIOLOGICAL TREATMENT
TECHNOLOGIES (SCS, 1980)

System trains are required for nearly all waste management operations (e.g., anaerobic → sludge dewatering → land farming; aerobic → sludge dewatering → incineration) and these costs are cumulative for waste management operations. The management of sludge can account for up to 50 percent of total treatment costs (Bryan, 1981a). In addition, cost factors based upon flow rates (e.g., $/1,000 gals) can be misleading in comparing different types of waste or waste streams. A more appropriate factor would be in cost per mass of waste destroyed (e.g., $/lb organic). Concentration of the waste, or the percent of contaminate in water, significantly effects what principal treatment technology can be used and at what cost. Table III-3 presents data on the approximate cost per pound of organic material destroyed (or in the case of landfills, buried). This data, adjusted to 1981 dollars using general inflation rates (e.g., 8-11 percent per year) results in the approximate profiles as illustrated in Figure III-3 (for the specific treatment process only).

Conventional aerobic technologies are, of the three biotechnologies illustrated in Figure III-3 (aerobic, anaerobic, and land farming), the most expensive per pound organic matter destroyed. These high costs are primarily due to the large hydraulic volume or pumping requirements for treating the dilute waste and the cost of supplying air through mechanical aeration systems as the percentage of organic matter or oxygen demand increases. These are inversely related.

With concentrations of waste approaching 4,000-6,000 mg/l, the oxygen demand becomes so high that aeration is no longer feasible and anaerobic systems become more appropriate. Anaerobic systems have the potential to produce methane as a potential energy credit, and some anaerobic systems can operate at a profit if a fuels purchaser is available. Anaerobic usually treats waste from 0.5-10%.

Wastes at organic concentrations exceeding 8-10 percent are generally characterized as either highly soluble organic solutions, or as a cakey, oily, insoluble sludge material, the consistency of which precludes the uniform mixing for the contact of anaerobic bacteria and substrates. Particularly with sludges exceeding 10 percent organic, material dilution and biodegradation can be applied on land at roughly the same cost as anaerobic digestion per pound organic matter destroyed. Costs for land farming tend to vary, however, as hauling is required and with increasing concentrations the land required for materials dilution becomes a limiting, though site specific, cost factor. Thus land, hauling, and waste types are cost variables.

Both landfill and incineration are indirectly related to organic concentration and are inappropriate for wastes containing more than 75-85 percent water. Though water is a medium for nutrient exchange

Table III- 3

APPROXIMATION OF COST PER POUND ORGANIC
FOR EXISTING BIOLOGICAL TECHNOLOGIES (1981 DOLLARS)*

	Ref.$ Year	Technology	Type	Conc. (ppm)	$/1,000 Gal**	$/lb Organic**	Reference
1.	1979	Aerobic (AS)	phenol	10	0.44	5.5	Blum, 1980
2.	1979	Aerobic (TF)	phenol	2,800	12.30	0.61	Blum, 1980
3.	pre 1977	Aerobic (AS)	dilute	150^1	0.15-0.6	0.12-0.46	ADL, 1978
4.	pre 1977	Aerobic (AS)	strong	1,500^1	1.46	0.12	ADL, 1978
5.	1978	Aerobic (AS)	mixed^2	250-1,000	4.14	0.50-1.71	SCS, 1980
6.	pre 1977	Aerobic (AL)	dilute	150^1	0.15-0.45	0.12-1.80	ADL, 1978
7.	pre 1977	Aerobic (AL)	strong	1,500^1	2.9	0.23	ADL, 1978
8.	1978	Aerobic (AL)	mixed^2	250-1,000	2.9-3.5	0.33-1.66	SCS, 1980
9.	pre 1977	Aerobic (TF)	dilute	150^1	0.15-0.4	0.11-0.32	ADL, 1978
10.	pre 1977	Aerobic (TF)	strong	1,500^1	2.7	0.22	ADL, 1978
11.	1978	Aerobic (TF)	mixed^2	250-1,000	2.2-3.2	0.27-1.5	SCS, 1980
12.	pre 1977	Anaerobic (D)	sludge	50,000	14.6	0.036	ADL, 1978

(AS) Activated sludge
(TF) Trickling filter
(AL) Aerobic lagoon
(D) Anaerobic digestion
(UF) Anaerobic upflow filter

(1) Approximation of concentration dilute (150 ppm)/strong (1,500 ppm)
(2) Mixed organic waste, no concentration given (assumed 250-1,000 ppm)
(3) Does not include methane credit
(4) Interpolated from 100 percent
(5) Total chlorine must not exceed 10 percent or requires dilution with fuel oil. Cost escalates as fuel oil is required to concentrate watery waste's Btu value

*Excludes cost of sludge disposal and credit for recovery of byproduct material

**Adjustment to 1981 dollars-inlation factors 1976-8%; 1977-9%; 1978-9%; 1979-11%;1980-11%

Table III- 3 (Cont.)

APPROXIMATION OF COST PER POUND ORGANIC
FOR EXISTING BIOLOGICAL TECHNOLOGIES

	$ Year	Technology	Type	Conc. (ppm)	$/1,000 Gal	$/lb Organic	Reference
13.	1978	Anaerobic (D)	sludge	50,000	5.12-6.37	0.01	SCS, 1980
14.	1979	Anaerobic (UF)	soluble	10,000	1.2-2.5	0.015-0.03	Ragan, 1980
15.	1978	Land farming	sludge	15-20%	16-22/ton	0.04-0.07	Grove, 1978
16.	1976	Land farming	sludge	15-20%	4.7/ton	0.011-0.015	Lewis, 1980
17.	1978	Landfill	sludge	100%	134-412/ton	0.07-0.20	SCS, 1980
			sludge	50%[4]	"	0.13-0.41	
			sludge	25%[4]	"	0.27-0.82	
			sludge	10%[4]	"	0.67-2.06	
18.	1981	Landfill	solid	100%	15-80/ton	0.01-0.04	Etzel, 1981
				50%	"	0.02-0.08	
				25%	"	0.04-0.16	
				10%	"	0.08-0.40	
19.	1979	Landfill	phenols	25%	140/ton	0.28	Blum, 1980
20.	1978	Incinerator	combustibles	100%	600-654/ton	0.30-0.33	SCS, 1980
21.	1981	Incinerator	combustibles[5]	100%		0.02-0.08	Wukasch, 1981
				30%		0.08-0.24	
				10%		0.25-0.61	
				5%		0.50-1.20	

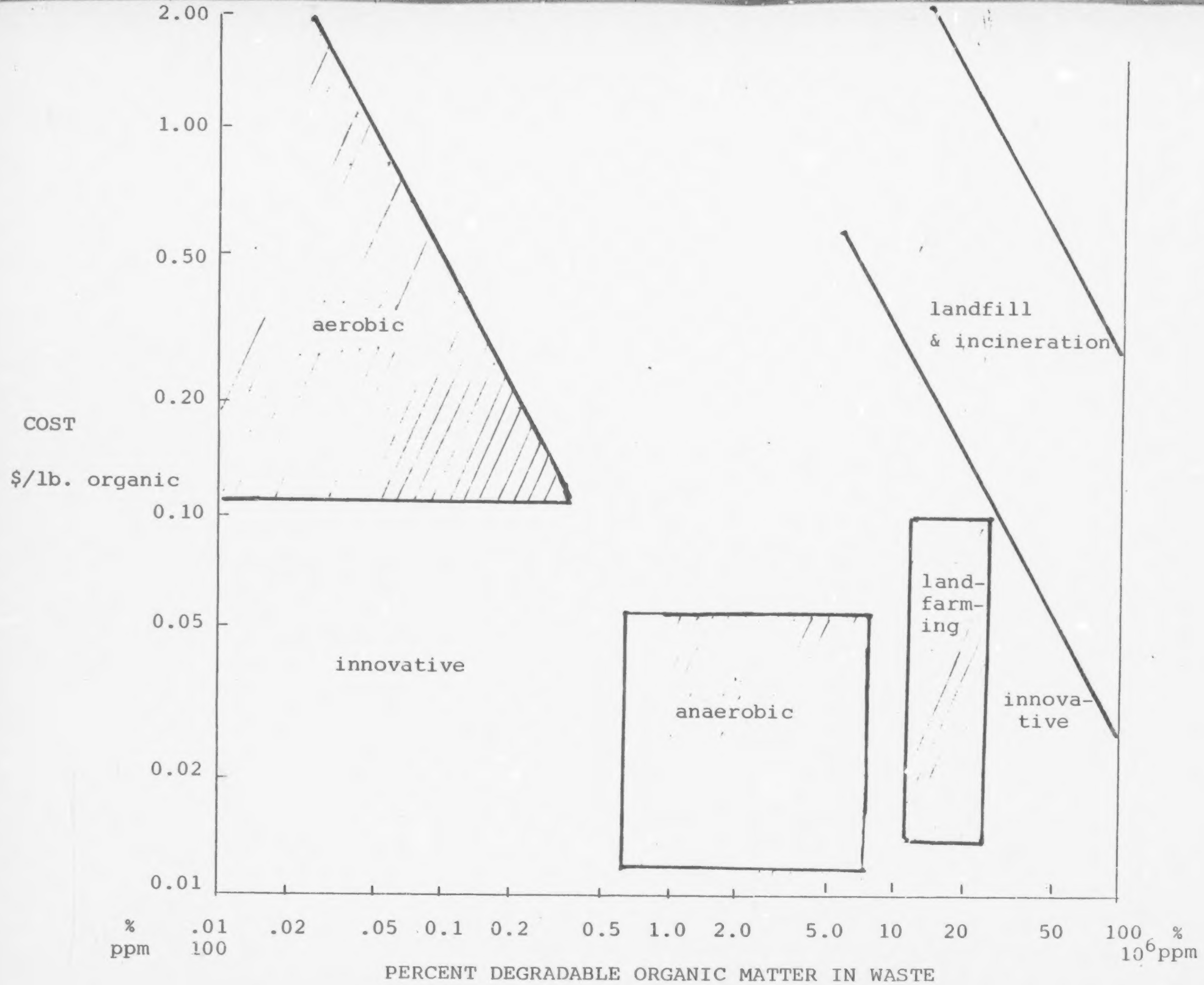


Figure III-3. COST/TECHNOLOGY/CONCENTRATION RELATION IN HAZARDOUS WASTE TREATMENT (from Table III-3)
(SOURCE: AmTech Consultants)

and cellular growth in biotechnologies, it is a costly burden for landfilling and incineration. In incineration, waste is preferred that will sustain its own combustion; however, with high water concentrations energy is consumed to vaporize water before the organic material in question becomes thermally oxidized. Consequently, fuel oil is required as an additive, which can become extremely costly for watery materials. For landfills, the cost of handling, hauling, and disposing of the water component becomes extremely cost-ineffective. (Furthermore, landfilling is not degrading the waste.) For highly concentrated solids or neat solutions, landfill or incineration is economical; however, when toxic waste is in solution or in sludge, system trains that include dewatering or solvent extraction can be expensive (Bryan, 1981a). One cost-effective thermal oxidation approach in industrial sludge management involves blending with clay followed by toxic destruction through kiln firing to produce ceramic building components (Alleman, 1980).

Figure III-4 illustrates that anaerobic digestion is the least costly mode of destruction of biodegradable materials and that dilute ($<$ 5,000 mg/l) streams might be concentrated, or concentrated ($>$ 200,000 mg/l) streams diluted to achieve minimum (1-10 percent organic concentration)* costs on a mass destroyed basis.

Finally, innovative manipulation techniques in biotechnology might result in enzyme systems or particular cell lines that could be used in all modes (aerobic, anaerobic, land farming) to increase the cost-effectiveness of existing biological systems. Innovative zones are noted in Figure III-4 where treatment costs could be less expensive.

C. Problems and Advantages of Biological Technologies

It is the principle of biotechnology to harness living organisms or their products in a mechanically engineered approach to control biological reactions. Consequently, there are not only problems that are engineering in effect but also problems that center on the biology, or the processes that internally control the system. The division is more distinct in biotechnology than in chemical, nuclear, or combustion technology as the biology is the driving force behind the process, more so than the mechanical engineering design can control for. Thus, there are commercial biotechnologies in use today where there is little knowledge of what is happening in the system, other than it is producing the desired effect (e.g., reported biodegradation of dioxins, Esposito et al., 1980). Nevertheless, as more becomes known about the biological parameters that control a process, a more theoretical rather than empirical approach can be used to reliably design a biological technology, similar to genetically engineering a bacteria. While we are knowledgeable about many external factors, such as temperature, concentrations, and toxicity, we lack the means for controlling the biological factors, such as genetically coded enzymes, interspecies competition and the fitness of individual populations in harnessed microbial systems.

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*Assuming concentration or dilution costs are inexpensive relative to alternative treatment techniques in the waste management process train

However, in spite of these deficiencies there are many advantages to using a biological process even with a lack of ability to control or fully understand the "mechanics" of the biological system. This is simply based on the cost-effectiveness that empirical data demonstrates in contrast to non-biological approaches, as discussed in the previous section.

Finally, however, there is particular concern from the public health perspective in assuring that a technology will be reliable when applied to toxic waste control. Biotechnologies for hazardous waste treatment pose a potential double risk in this regard, one being the risk associated with the toxicity of the chemical waste itself and the other concerning the spread of toxin-producing microbial life forms.

1. Operational Factors

Table III-4 presents general operating concerns associated with the use of biological technologies in treating hazardous wastes. As Table II-2 lists the factors contributing to a chemical's recalcitrance, Table III-4 presents the major engineering and biological factors that need to be considered in the design and operation of biological systems.

Table III-4

MAJOR OPERATIONAL CONCERNS IN
HAZARDOUS WASTE TREATMENT WITH BIOTECHNOLOGIES

Engineering	Biological
Kinetics	Waste Composition
System Aeration	System Upsets
Bacteria/Waste Contact	Microbial Viability
Stripping of Volatiles	Biological Competition
Emulsifying/Solubilizing	Nutrient Balance
Flow & Waste Equalization	pH, Temperature
Monitoring	Inorganic Salts
Sludge Management	Toxicity
Solids Plugging	Recalcitrance
Gas Pockets	Monitoring

(SOURCE: AmTech Consultants)

The development of operational parameters or technology that address these concerns can be major problems in biological waste treatment systems, and many of these factors can limit the selection of a biological process if a practical solution cannot be obtained. The following discusses some of the problems associated with developing these operational parameters or associated technology.

a. Engineering

Kinetics. It is necessary to establish the rate at which bacteria destroy the waste so as to avoid under- or over-designing a system. From this information mean cell retention and hydraulic retentions can be calculated. The parameters will vary for differing microorganisms and substrates and are particularly important for genetically engineered microorganisms or systems requiring bioaugmentation. The kinetic coefficients that are important are:

- Growth yield coefficient (Y)
- Waste concentration at maximum utilization rate (K), 1/2 maximum rate (K_s), and 1/2 inhibition rate of waste utilization (K_i)
- Microorganism decay coefficient (K_d)

These factors are difficult to establish for mixed wastes, mixed cultures, or low concentrations of toxic organics due to nonlinearity of degradative activity and co-metabolism effects.

Aeration. If a system is operated aerobically, oxygen demand must be satisfied sufficiently to maintain an excess of free oxygen in the treatment process. This can be difficult and costly for sludges or highly concentrated wastes because energy is required for pumping and both sludges and highly concentrated wastes have high oxygen demands.

Waste/bacteria contact. Biological systems perform optimally when a maximum surface area of bacteria is exposed to the waste. For dissolved waste, fluidized, fixed, or rotating inert media can be used to maximize contact with the bacteria growing as a film on the media (e.g., trickling filter). Sloughing or loss of the bacteria from the media is a problem. With sludges, bacteria will grow suspended directly on the substrate and maximum performance is obtained with well mixed systems containing small particles. Biological treatment of insoluble substrates occurs in a similar manner as sludges and uniform adsorption of the insoluble substrate on suspended solids provides for maximum utilization of treatment space. Designing biological systems for sludges and insoluble organics are major problems and emulsifiers are frequently required.

Stripping. Many toxic chemicals, e.g., vinly chloride, can be lost to the atmosphere through volatilization. It is important to establish the rate of volatilization in the presence of suspended solids to provide for proper design and to control the vapor to the extent that it will not cause an occupational or public health hazard. The rate of volatilization can be affected by the presence of suspended solids, thus empirical data rather than theoretical vapor pressures or Henry Law constants are required.

Emulsifying/solubilization. Pretreatment or mixing of insoluble organics to render them soluble or emulsified on fine particles is necessary for optimum biodegradation. Biological activated carbon or mucopolysaccharides secreted by bacteria are examples of emulsifiers that adsorb insoluble organics and simultaneously allow for bacterial growth on fluidized suspensions.

Flow and waste equalization. Rapid rises in the type or concentration of wastes can create an imbalance in biological system performance contributing to upsets. Equalization chambers and emergency holding basins are necessary for most biological systems where flows and waste compositions vary. Designing capacity to prevent shock-loading can be difficult to predict and their installation costly.

Monitoring. Continuous monitoring of waste concentrations and operating conditions are necessary to predict system upsets and corrective actions. Continuous monitoring of diverse types of toxic wastes or buffering levels can be time-consuming or costly.

Sludge management. Sludge dewatering, handling, and ultimate disposal are always concerns and their ultimate disposal is dependent upon the efficiency of the treatment system to degrade the toxic chemicals.

Plugging. The clogging of bed media with sloughed particles or suspended solids in the waste creates problems in design and operation. For anaerobic systems, gas pockets can also create problems in maintaining constant pressures and utilization of effective reactive space.

b. Biological

Waste composition and concentration. Knowing the type of wastes and their optimum concentrations for biodegradation are of major importance. Establishing the microbial kinetic coefficients of bacteria for differing substrates are the primary determinants in biological system design or in bioaugmentation operation maintenance methods. No common units of bacterial formulation substrate activity have been developed for commercial bioaugmentation products.

Upsets. Biological systems are acclimated and sensitive to specific types and concentrations. Rapid increases in concentration or changes in composition can lead to upsets or toxic inhibitions.

Enzyme interference. The breakdown of highly complex wastes are usually dependent upon the rate of production and activity of specific enzymes synthesized by the bacterial community. These enzymes can be induced by the presence of a substrate or metabolite and also repressed. In addition, their activity can be inhibited by a structurally similar substrate or by the binding of the enzyme to another substrate. Maintaining a continuous production of functioning enzymes is the objective of well acclimated biological treatment systems; however, their repression or inhibition probably accounts for the presence of many toxic chemicals in waste treatments because they are inactivated. Maintaining enzyme production and preventing competitive and non-competitive inhibitions are major problems in biological treatment systems and should be overcome by genetic engineering and bioaugmentation. For example, promoter optimization or mutations in the genetic regulatory sequence can cause continuous and enhanced production of enzymes overcoming genetic repression. Increased concentrations of enzymes can also saturate inhibitors and allow for more substrate specific activity. Bioaugmentation can in a like manner provide for the excess enzymes. Genetic engineering can affect the structure of enzymes and allow for new substrate specific conformations.

Microbial viability. Increasing the fitness of major enzyme producing organisms is a problem in biological systems. A highly effective organism may be extremely well suited for growth as a pure culture on a specific toxic substrate, but could be inhibited in the presence of other substrates or organisms.

Biological competition. Unwanted microorganisms or algae may grow in the system competing for food sources, reactor space, and interfere with the operation of the system. Bioaugmentation may allow for more tolerant organisms and those that preferentially compete with the unwanted variants for food sources, thus diminishing their presence. Aseptic systems may also be used.

Nutrients. A carbon/nitrogen/phosphorous balance plus essential trace elements are required for proper biological growth. Aerobic systems require more nitrogen and phosphorous nutrients than anaerobic systems. A ratio of 750-75/5/1 is usually adequate with the higher value (750) being the upper boundary for anaerobic. Nutrient supply could be costly for toxic wastes concentrated with hydrocarbons.

pH, temperature. Changes in pH and temperature can cause an upset in biological systems. The addition of buffering agents and maintaining adequate temperatures can be costly. Environments such as the Northeast and Northern Midwest preclude the maximum use of biological systems in open lagoons or ponds through the winter season.

Inorganic salts. Though biological degradation can occur in salt as well as fresh water, sudden changes in salinity can affect the osmotic regulation of bacterial systems and inhibit performance. In systems where water is totally recycled, the return flow of mineralized products from the degradation of highly chlorinated organics could build up high concentrations of Cl and buffering salts. Either make-up water or demineralization of recycle streams would be required.

Toxicity. Certain organics and inorganics could be present in the waste water at high concentrations inhibiting biological performance. For example, sulfate converted to sulfides in anaerobic reactors and cyanides at high levels can be toxic. Most all organisms have a concentration plateau where optimum substrate utilization occurs. Free enzymes or more physiologically fit organisms may tolerate toxicity effects.

Recalcitrance. Some wastes can be highly recalcitrant to biodegradation, for example, highly chlorinated polychlorinated biphenyls and dioxin . Nevertheless, bacterial and plasmid systems may evolve or be developed for their degradation. Some compounds may also be more readily consumed under anaerobic or aerobic conditions than others, or a combination of the two. This is generally dependent on the catalyzing enzymes requirement for free oxygen and enzymes frequently given the term oxygenases. Aromatic compounds, or ring structures that are highly substituted, are more readily degraded by aerobic systems or those requiring free oxygen, presumably due to the requirement of oxygenases to cleave the ring. On the other hand, anaerobic systems have been demonstrated to break down highly complex ringed compounds under thermophilic conditions, e.g., lignin, and reductive dechlorination of aliphatics and aromatics. Thus, though chemical recalcitrance is apparent under many conditions there is no general rule for determining what is biodegradable and what is not.

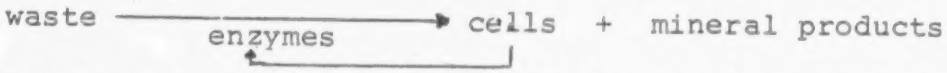
Monitoring. Monitoring of chemical toxic waste characteristics in effluent and sludge are extremely difficult, time consuming and costly. Biological systems are best operated on isolated waste streams where a consistent quality and quantity are assured. Standard cell lines developed for bioaugmentation could be used to screen variable sludges and effluent for biodegradability and those stock cultures used to start and maintain batch treatment processes or augment continuous flow systems when the effluent or sludge is fed.

c. Major Problems

The major problems associated with the use of biological technologies are concerned with parameterizing or developing ancillary technology that address the engineering or biological factors prior to commercial development. To summarize, these problems are focused not only on finding an appropriate organism or product to promote degradation of the waste but also developing the engineering criteria and technology hardware needed to design and operate the system.

2. Advantages of Biological Technologies (Table III-5)

The principal advantage in using a biological technology is that the microorganisms perform ultimate degradation, or destroy the waste while using it as an energy source. Unlike physical/chemical systems, biological processes are like self-programmable computers, turning on or off, or naturally selecting for genetic codes to produce the enzymes required for destroying the waste. Naturally, those cells that produce the enzymes are more fit to survive and multiply in the environment containing the waste as a food source. If the waste changes in type or is removed from the environment, those cells in turn become less fit for survival. This is an advantage when one is concerned about the spread of a genetically engineered bacteria through the open environment. Genetic engineering of bacteria can help to speed up the natural evolutionary process and provide attributes to cells to help them survive under otherwise hostile conditions. Bioaugmentation, or the addition of organisms to treatment systems, allows for the increased concentration of enzymes that bacterial growth alone may not be able to provide, and enables the enzymes continual presence when the survivability of the microorganism is low. Bioaugmentation is analogous to the makeup of spent catalysts in chemical systems, but is distinctly different and advantageous in that the biological catalysts (enzymes) can be continuously produced internally at the expense of the waste it is degrading.



Biological systems also have the advantage of treating relatively weak to concentrated solutions; however, their efficiency in treating highly concentrated solutions diminishes, unless the treatment process provides for some form of material dilution, as occurs in land farming or co-disposal in waste water treatment plants. The costs for incinerating or biologically treating highly concentrated neat solutions can be comparable; however, for weak or dilute solutions biological processes are more effective if the material is biodegradable. For example, Chakrabarty (1981) demonstrated the efficient detoxification of 2,4,5-T when diluted to 10,000 ppm in garden soil.

Biodegradation is a phenomenon that is observed throughout the earth--in oceans, lakes, rivers, soils, and ground waters and in symbiotic relation with higher plants and animals, such as with enteric bacteria in the intestines of man or microbes degrading wood for termites. Microbes are found in nearly every conceivable environmental niche, including hot geysers and volcanoes, the depths of the oceans, and utilize the chemicals present for a nearly infinite

Table III-5

ADVANTAGES OF BIOTECHNOLOGIES

- Uses waste as a food source
- Adaptable to extremes
- Naturally enfeebled by being specific
- Genetic Engineering
    - speeds adaptation process
    - provides unique characteristics
    - increases quantity/quality of enzymes
- Bioaugmentation
    - compensates for poor growth and survival
    - similar to adding catalyst but better; allows for continuous production at expense of waste
- Cost-effectively treat weak and concentrated solutions
- Optimizes natural processes
- Plasmid transmissibility

(SOURCE: AmTech Consultants)

number of biochemical reactions. It has even been suggested that high temperature thermophilic organisms may be responsible for the disappearance of waste injected into deep wells (Schroy, 1981). Bacteria can produce energy from waste, such as methane and ethanol, chemical feedstocks, and can mineralize heavy metals as insoluble sulfides or release them into solution. Optimizing the conditions to promote the microbe's waste degrading activity is an objective of environmental engineering and can occur in fermentors, tanks, landfills, lagoons, ground water, and soils.

Because of the attributes of biological processes, once the conditions have been established to optimize waste degradation, the costs can be considerably less than for other chemical/physical approaches.

One major advantage in genetic engineering is that genetic codes might be structured for the complete mineralization of a chemical on one plasmid, and this plasmid in turn, being transmissable to other microorganisms in the waste treatment process, making the whole system primed for that particular type of waste.

3. Public Health Risks

The main public health risks associated with the use of biotechnologies in general and applied genetics in particular are concerned with the release of a pathogenic organism into the environment. Biological technologies are in widespread use throughout the pollution control industry, and though many studies have focused upon the potential spread of disease-causing organisms from their aerosols, no significant effects on public health have been noted (Etzel, 1981). Likewise, careful monitoring in the recombinant DNA industry for the health of laboratory workers has revealed no unusual effects (OTA, 1981).

Table III-6 summarizes the health risks of concern. In the treatment of hazardous wastes biologically, the concerns focus on natural pathogens in pollution control, the potential use of pathogens in biological formulations, and their inadvertent development through applied genetics. Furthermore, there are concerns about overall risk and methods to contain the possible spread of microorganisms from treatment systems should pathogens be inadvertently innoculated or cultured within the process.

a. Natural Pathogens in Pollution Control

Pathogens are types of aerobic and anaerobic bacteria, protozoa, and viruses. The common pathogenic bacteria originate from human excreta and include Salmonella typhosa, Shigella dysenteriae, Vibrio comma, and Vibrio cholerae. A major public health concern is the transmission of these organisms through water from the feces of humans, and less pathogenic and more ubiquitous coliform bacteria of enterobacteriace, escherichia and aerobacter, have been used for monitoring. The mode of action of most pathogenic bacteria is through

Table III-6

PUBLIC HEALTH RISKS ASSOCIATED WITH THE USE OF BIOTECHNOLOGIES IN HAZARDOUS WASTE CONTROL

MAJOR HEALTH RISKS OF CONCERN

A. Natural pathogens in pollution control systems
B. Pathogens in biological formulations
C. Inadvertent cloning of exotoxin in rDNA
D. Containment in use

A. NATURAL PATHOGENS IN POLLUTION CONTROL SYSTEMS

1. Bacteria, viruses, protozoa
2. Sources usually from human excreta
3. Possible risk in co-disposal of toxic waste with sewage-aerosols as pathway
4. Conventional sewage pathogen control

B. PATHOGENS IN BIOLOGICAL FORMULATIONS

1. Not an edible product, thus low exposure potential
2. Bacteria acclimated to toxics have low open survival
3. Appropriate contained use in waste treatment
4. Pathogen contamination requires screening procedures
5. Greatest concern in production and handling

C. GENETIC ENGINEERING AND TOXIN PRODUCTION

1. Product used as biological formulation
2. Pathogenic host or exotoxin insertion would be inadvertent and likelihood very low
3. Natural plasmid evolution has low probability of toxin development except for natural pathogens

D. RISK AND CONTAINMENT

1. Risk is low, thus containment concerns are low
2. Testing or screening of products should be performed
3. Normal controls as practiced with pathogens in sewage treatment applicable, e.g., disinfection, irradiation
4. Enfeebling organism through rDNA unnecessary if toxic waste required for survival
5. Use in land farming, spills, potable aquifers, are less confined than on-site fermentations, lagoons, or reactors

(SOURCE: AmTech Consultants)

the release of a toxic matabolite (toxin) rather than the organism per se. There are endotoxins and exotoxins produced by the cell. Exotoxins are released by the bacteria into surrounding fluids, are proteinaceous, and very potent. Endotoxins are lipopolysaccharides, an integral part of the cell wall, are less potent than exotoxins, and only exert their toxic effect when the cell wall is disturbed (Van Heyangen, 1970). There are some endotoxin producing microorganisms that are used commercially as insecticides. Because exotoxins are protein based, there is a concern for their production directly from genetic material.

Bacteria are about 0.2-5 microns in size, protozoa considerably larger, and viruses considerably smaller, 0.015-0.3 microns. The latter, viruses, can contain only RNA, and invade a host cell of a higher organism and essentially take over the genetic transcription for its own reproductive purposes (interferon is associated with the genetic formation of a protein by the host cell to combat this virus). There are many disease-causing viruses which are of particular concern to public health authorities. Viruses generally do not survive very long in the external environment, not having a host cell to colonize compatible with transmission to man, but vectors, such as certain insects, can thrive in external environments, increasing the probability of viral disease transmission when hygienic practices for insect control are not followed.

Viruses continue to be a concern to sanitary engineers in the treatment of municipal waste water and disposal of sewage sludge (Bryan, 1981b). Also, concern of the use of a cancer-causing virus in non-human cells as a vector for the recombination of DNA has nearly precluded the use of viruses in genetic engineering (OTA, 1981).

Protozoa, such as _Endamoeba histolytica_, are also of public health concern but are controlled with proper disinfection of both drinking water and sewage plant discharge, as are bacteria. The size of the larger bacteria and protozoa nearly preclude their transmission through ground water with low soil porosity (Keely, 1981).

Generally, outside the environment of the human, these pathogens are relatively unfit for survival, and their reduction rather than propagation occurs in conventional waste water treatment. Because human excreta is the source of many pathogens, the biological treatment of toxic or hazardous industrial waste should not be of concern for the spread of these natural pathogens, unless toxic wastes were co-disposed, for example, into a municipal sewage treatment plant, or with municipal sewage sludge in landfills or on land farms.

b. Natural Pathogens in Biological Formulation

The intentional use of biological cultures, produced under controlled laboratory conditions for augmenting the degradation of toxic materials, poses no more and probably a less risk than, for example, formulations or contamination in the dairy and food industry where the product is used for human consumption. The main concern of formulations for bioaugmentation purposes is the use of a pathogenic organism, or, more likely, contamination of the product by a pathogenic organism. Unlike the use of microbes or cultures in the food industry (yogurt) or in contamination of food products (Clostridium botulinum) the formulation is for use in waste water or hazardous waste facilities, not for consumption by humans. This use is in a very appropriate environment from the hygienic perspective (a hazardous waste treatment facility).

Furthermore, the health risk of the toxic waste itself is probably far greater than that of the organism living on the waste, and once that substrate is absent, the microbe will either die (Chakrabarty, 1981) or revert to a more normal metabolic process (McDowell, 1981). With the absence of the substrate the microbe was conditioned to, it would most likely be much less fit for survival in comparison to other members of the microbial population that are naturally present. Therefore, the use of microbial formulations should pose very little risk to public health when used according to the manufacturer's instruction in waste treatment facilities and are screened for pathogens or contamination by pathogens. Still, in such an event of pathogenic contamination, the main concern would not be in the waste treatment facility but in manufacturing or at the facility where handling of the material occurs.

It is somewhat ironic that there is a public health concern about the use of microorganisms in pollution control systems, when these systems are the principal means for developed civilizations to contain and reduce the existence of pathogenic bacteria from humans and to treat toxic pollutants. Nevertheless, approaches to pollution control which consider the use of microorganisms, genetically engineered or not, are often cited for the fear of the spread of some known or unknown disease, the possibility of mutations, or the formation of toxic metabolites (Freestone, 1981).

Packaged biological formulations acclimated in the presence of toxic chemicals, however, should not be used for animal consumption as they may contain traces of the toxic material in question, though otherwise could be considered as single cell protein. They should also be handled with care, and if in powdered form, precautions should be taken to avoid breathing any dust generated as it may illicit an allergic response in sensitive individuals or contain a general irritant. The handling and use of biological formulations should be indicated by the manufacturer, and responsibility clearly identified.

Though pathogens are unlikely to be present because the bacteria for hazardous waste treatment are selectively adapted and acclimated for growth on a specific sole source of carbon, products should be tested for the presence of pathogenic organism contamination.

This testing is currently required when a product is used in waste water from several food industries (e.g., meat, poultry and egg product establishments) by the United States Department of Agriculture. The manufacturers of LLMO (Liquid Live Micro Organisms) perform routine screening for *Salmonella* (GESC, 1981), as well as for *Shigella*, *Streptococcus*, *Staphylococcus*, and sometimes *E. coli*.

The types of microorganisms present in two commercial formulations are presented below (GESC, 1981; McDowell, 1981). Neither of these formulations contain organisms associated with major pathogenic disease and should pose no significant health risk in their use.

LLMO Formulation

Aerobacter Aerogenes
Bacillus subtillus
Nitrobacter winogradski
Pseudomonas stutzeri
Pseudomonas denitrificans
Cellulomonas biazotea
Rhodopseudomonas pulustris

Hydrobac Formulation

Pseudomonas - 3 species
Nocardia - 2 species
Bacillus - 5 species
Micrococcus - 2 species
Saccharomyces 1 species

The LLMO formulation above has been tested in mice, through oral administration and intravenous injection for toxicity (GESC, 1976). In each case no effect was noted on the growth rate or mortality of mice in comparison to a control population.

c. Genetically Engineered Organisms in Pollution Control

Genetically engineered organisms used for pollution control should pose no more of a public health risk in the transmission of infectious disease to man than those described for bioaugmentation, but there are two possible exceptions. These are (1) when a pathogenic host is used as the organism to harbor the plasmid or recombined gene, and (2) when the genetic code responsible for a disease or exotoxin is introduced into a ubiquitous microorganism.

In regard to the first issue, insertion into pathogens should never be conducted unless for medical research or disease prevention. It is highly unlikely that a microbiologist or manufacturer proposing to use a cell line in pollution control would knowingly use a pathogen for such applications in the open environment. Use of *E. coli* does pose some concern, however, and though not a cause of serious infectious disease, its use as a host organism could be at slightly greater risk than other non-enteric bacteria (e.g., *Bacillus*) used in rDNA. Insertion of genes into enteric bacteria does present the opportunity for the clone to propagate in the human intestinal tract, thus when they are used and are not pathogenic, good laboratory practices should be considered the rule when handling the material.

The second possibility, the insertion or cloning of a gene which expresses the formation of an exotoxin, could be done knowingly in a field outside of pollution control for a variety of medical research reasons such as antibody production. In pollution control toxin production from rDNA would have to be inadvertent and because the organism would have to survive through the adaptation process of selection on sole sources of toxic hydrocarbons, its likelihood is decreased. Finally, the state-of-the-art in applied genetics in hazardous waste control is oriented toward natural plasmid evolution for cloning. As previously discussed, plasmids are also noted for the evolution and interspecies transfer of genetic material that codes for specific enzymes that destroy or metabolize material toxic to the cell. Thus, due to the nature of plasmids, the manipulation of plasmid DNA in-vivo should pose insignificant risk of inadvertent toxin cloning.

There is a third but highly remote possibility equal to the probability of natural spontaneous mutation, and that is the mutation of a formerly non-toxin producing gene sequence inserted into a non-pathogenic organism that in turn produces a toxin. The probability of this occurring is no greater than the same occurring for organisms in the natural environment, and should it occur the cell itself may become debilitated and less fit for survival on the waste in question.

Finally, there is the concern for a bacteria or a genetically engineered organism that degrades a toxic chemical to contaminate actual supplies of the same commercial (or natural) material. First, this is unlikely as most organisms isolated require controlled, usually well aerated, conditions for extended growth, at relatively low concentrations (< 2 percent of the product). Commercial products are relatively pure, are not aerated, and the type we are concerned with is contained due to the potential health impact as a toxic material. Second, the organisms of concern would have to be transmitted through the environment to production or storage facilities, a probability highly unlikely. Third, if such growth on commercial products were to occur it is thermodynamically remote for such contamination to consume very much product in the product's lifetime. Fourth, the transfer of products from one processing stage to another for upgrading, precludes survival through most such industrial systems.

d. Risk and Containment

The Guidelines for Research Involving Recombinant DNA Molecules stressed that the level of containment was to match the estimated risk. Risk is generally determined as the probability of an event occurring times its consequence. Though, as discussed above, the probability for a public health or welfare event to occur through rDNA organisms is extremely remote, the consequence of such an event, should it occur, might be significant due to the use of the material in the environment, though in well contained streams.

Therefore, as with any newly manufactured product suspected of toxicity or material impact, a rapid screening procedure or animal study should be considered with the potential to identify any toxin effects on man (or animals), as in the premanufacturing notifications of the Toxic Substances Control Act. As indicated above, these likelihoods are low for bacterial formulations produced for pollution control of hazardous waste, and the cultures are used in the most appropriate open environment, waste treatment systems,

Should controls ever be warranted the same disinfection procedures that are normally applied to control the spread of pathogens could be applicable at waste treatment facilities such as chlorination of water or irradiation of sludge (Bryan, 1981b).

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IV. BIOTECHNOLOGY APPLICATIONS IN SELECTED INDUSTRIES

When one looks at the biotechnology involved in the treatment of hazardous wastes, particularly as it would apply to the electroplating, pesticide, leather tanning, and chlorinated hydrocarbon types of production industry, it is obvious that certain practical rules have to be set. One of the first questions that arises when one uses biotechniques is whether they should be of the aerobic or anaerobic type or whether they should be a combination of the two. In general, the use of anaerobic systems with ring compounds and particularly with ring compounds that are highly chlorinated is difficult. In the case of compounds that do not contain benzene rings, whether they be chlorinated or not, the strength of the waste is ususally the determining factor as to whether anaerobic or aerobic means are used. The next question that must be considered is whether the treatment should be done on- or off-site. In the case of electroplating wastes, the chances of using any biological or biotechnological means of treatment on-site are essentially nil. The wastes do not contain significant amounts of organic materials and therefore are not readily amenable to any biotechnology. In the case of off-site treatment, it is possible that, by add-mixture with other types of wastes, the toxicity can be reduced or overcome when enough other carbon sources are available so that the wastes can be treated in a proper manner. The question still, however, remains as to what to do with metals that result in the sludges from this type of treatment and ultimately they must be either recovered or disposed of. The question of recovery is at the present time a mute one since the recovery of mixed metals has never proven to be economical, but the recovery of individual metals seems to be gaining enough interest to be of concern.

A general look at whether the biotechnology could be applied onsite would suggest that the wastes from tanning, certain restricted wastes from pesticide manufacture, and certain restricted wastes from chlorinated hydrocarbon production or use could be done directly onsite if the concentrations are not too high. This treatment would either result in a concentration of hazardous constituents or possibly more realistically the destruction of them by biological means. For small generators, the off-site treatment of wastes would be in order for the more concentrated and toxic pesticide materials and for the more concentrated and toxic chlorinated hydrocarbons (e.g., still bottoms). In these particular cases off-site treatment would be effective because it may require economies of scale and highly sophisticated pretreatment or fermentation systems and possibly admixture with other wastes. However, these materials could be properly handled onsite if similar technology and other wastes were available and economy of scale did not offset transport costs.

A. Electroplating

When one thinks of the wastes originating from the electroplating industry the question of biotechnology or biotreatment never seems to gain much favor because of the toxicity and because of the absence of organic materials. While this is always the case, there are systems that have been reported such as the work by Dugan and Pickrum (1972) that have shown that the biological systems have an ability to ion exchange or adsorb heavy metals onto the surface of bacteria and thus render the wastes non-toxic. The sludge resulting from these processes is still a problem, however, because of the presence of heavy metals, but the cyanide content of the waste is not a problem because it has been metabolized through aerobic means into carbon dioxide and nitrogen and thus been destroyed. It is obvious that this type of treatment would require a carbon source since Dugan and Pickrum report that this work was mainly done by mixing plating wastes with the wastes from municipal sewage. As to the use of biotechnology for the destruction of cyanide wastes, this is a well established fact that by aerobic means of enculture enrichment it is possible to destroy low to moderately high levels of cyanide. These levels are reported to be as much as 50 to 100 mg/l of cyanide if the cyanide is fed to the system on a routine basis. Gaudy, et al. (1980) and Rapean, et al. (1980) have shown work both full-scale and laboratory on the biodestruction of cyanide in treatment systems using biotechnology applications. It is again emphasized that in the treatment of the wastes from electroplating by any type of bio-system that it would not be possible to do it on the site plating plant becasue of a lack of a carbon source. It is also questionable whether or not any great amount of transportation of diluted wastes would be possible and therefore some means of waste concentration prior to their transport to a common location where a carbon source would be available would have to be part of any scheme for the handling of these types of hazardous wastes.

B. Pesticides

It is uncommon for pesticides to be produced within the main complex of a chemical plant and therefore these wastes can usually be segregated from the overall plant's waste stream. A further segregation is possible from the primary and intermediate production steps to obtain relatively consistent quality and quantity of waste. Thus, by means of waste segregation and pretreatment, these highly toxic materials are usually concentrated and can be handled onsite by such processes as chemical destruction or incineration. The remaining toxic wastes which are of a lesser degree of toxicity or hazardous type are generally handled on the basis of a biotreatment system which is of an aerobic variety. Separation of the wastes into the highly toxic or hazardous and the less toxic or hazardous streams is a standard technique used in the industry and discussed to a great extent in the development document for the pesticides industry (EPA, 1976). Work which has been done for a specific pesticide manufacturer has shown that this type of technique is a very viable one (Etzel, 1981). In this case incineration of the highly hazardous or toxic wastes makes it possible to use biotreatment of a aerobic variety and to successfully destroy the hazardous waste in the less concentrated material. In order to do this, however, it must be recognized that the biological system must carry a very high cell mass and must be completely mixed in order to minimize toxicity problems and to assure that no concentration of materials reaches a toxic or hazardous level to the microorganisms.

Some of the more promising methods in development consider use of biological manipulation methods, bioaugmentation and free or immobilized enzyme systems.

Using a commercial strain of bacterial formulation, Phenobac^{TM}, and comparing it to laboratory scale aerated test systems innoculated with soil from biodegradation test plots and sewage, Wachinski et al. (1974) were able to demonstrate 74 percent destruction of 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) from 1,380 ppm in 14 days in contrast to 47 percent and 13 percent biodegradation from the other respectively cultured systems. Though never fully tested by Wachinski due to the concern of residual dioxin destruction, Figure IV-1 illustrates a proposed pure oxygen biological treatment system for treating the Air Force's 2.3 million gallon stock pile of dioxin contaminated 2,4,5-T. As illustrated, a neat solution is mixed with sewage water and is reacted in a closed, air filtered, pure oxygen-activated sludge plant. The effluent is settled and the sludge anaerobically digested with the super-natant either receiving further aeration or carbon polishing prior to discharge. The sludge in turn is land farmed. Should a microbial strain be developed for treating both dioxin and 2,4,5-T, such a system design might be applicable for treating this or similarly related concentrated toxic waste materials.

Münnecke et al. (1976-77-79) has performed an extensive amount of research on the use of a free or immobilized enzyme, parhydrolase, in detoxifying organophosphate pesticides. The enzyme which is quite stable in extracted form shows a broad substrate specificity to organophosphates (e.g., Parathion) and could be used cost-effectively in detoxifying both commercial product residuals or plant wastes containing the material if enzyme production costs could be reduced.

Another application of biotechnology in the industry involves end product substitution to offset wastes generated in the manufacture of chemical pesticides. In these cases microorganisms pathogenic to specific pests can be used as an application method. For example, Abbott Laboratories produces and markets various biological pesticides.

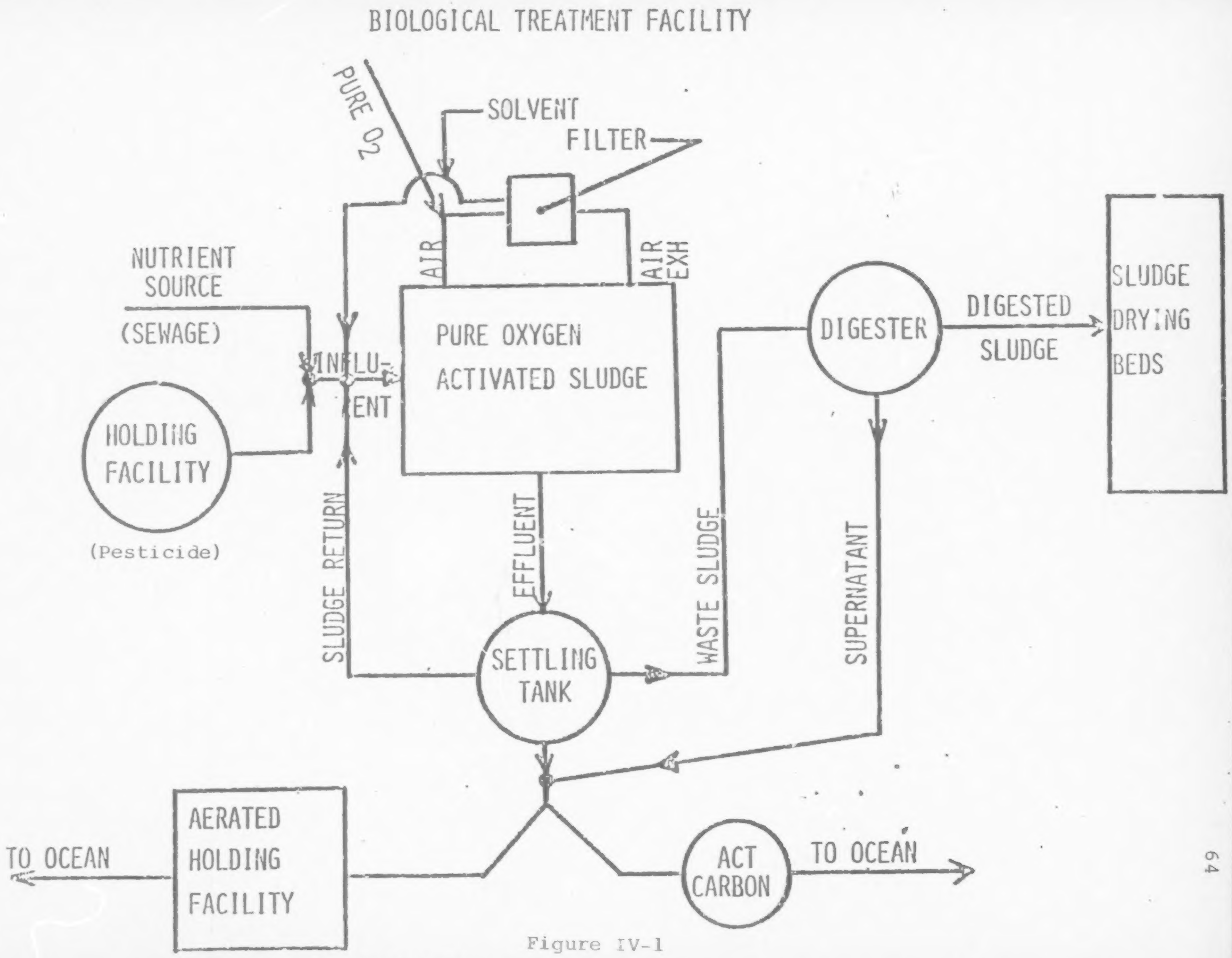

Figure IV-1

PROPOSED BIOLOGICAL TREATMENT FACILITY FOR 2,4-D AND 2,4,5-T (Wachinski, 1974)

Bacteria, such as Bacillus thuringiensis (DIPEL), can be applied in a spore form to garden plants providing an endotoxin specific to lepodoptera larva. Similar products, such as VECTOBAC, are Bacillus subvariety specific to mosquito larvae (Couch, 1981). There are many other biological methods on the market for home and garden use (Batra, 1982).

C. Leather Tanning

When one looks at the tanning industry, it is obvious that the only type of hazardous waste which can result is that from the chrome tanning of leather since there are no hazardous organic materials used in the vegetable tanning area. The EPA lists leather tanning for its chromium and lead as a hazardous waste. In this respect, this discussion of the use of biotechnology for the treatment of tanning wastes will be limited to a discussion of the hazardous materials that can result when leather is tanned by the chrome process. The two materials of most concern are chromium and sulfide. There is a slight possibility of disease transmission from the wastes, but this is so slight that it is really never considered to be of a significant nature in the treatment of wastes from the tanning industry. In many cases, the development document (EPA, 1974) shows that the wastes from tanning are treated separately from beam-house waste. This is because of the high strength of the one type of waste versus the presence of the chromium and sulfide in the other. Pretreatment of these wastes to remove the chrome and the sulfide has proven to be successful onsite for almost all tanneries, but the resulting sludges which are quite voluminous are still hazardous because of the presence of chromium and sulfide and ultimately must be disposed of off-site at some point in time. More recent work done by Nemerow (1981) showed that, if he combined the two wastes together, he could achieve treatment without having to remove chrome or sulfide and that by doing this it was possible to produce an acceptable quality effluent and the resulting sludge was considerably less hazardous because the sulfide had been destroyed and only the chrome remained in the sludge. To do this, however, he also made the tannery practice inhouse recovery of both chrome and sulfide to the maximum extent possible. Except for the problems resulting from the presence of chrome in the sludge from the tanning industry, it would seem that this industry does not have a significant hazardous waste problem that cannot be solved onsite with the application of existing aerobic technology and good internal housekeeping.

D. Chlorinated Hydrocarbons

Since this category of materials is not unique to one industry but is a broad spectrum type coverage it must be emphasized that the materials could originate in petrochemical plants, typical chemical plants, pesticide production plants, etc. The same general rules as previously described, that being the low chlorinated non-ring compounds should be able to be treated very readily onsite with a biosystem which would result in destruction and thus the production of no hazardous wastes at all. If, however, the compounds contain multiple rings and have high degrees of chlorination, then the likelihood of being biotreated are reduced and they would probably have to be destroyed by some physical or chemical means. Ring compounds that have lower degrees of chlorination such as the lower chlorinated polychlorinated biphenols and pentachlorophenol have been successfully treated by aerobic means with a resulting destruction of their hazardous nature because of the biodestruction of the materials themselves. To substantiate this recent work (Etzel, 1981) involving the destruction of PCBs that were of the 12-42 and 12-48 variety have shown better than 96 percent destruction of the PCB across an aerobic biological treatment system which has a long sludge age and a well-mixed aeration basin. These data are quoted so that it can be shown that with proper evaluation and testing it is possible for certain types of chlorinated hydrocarbon to be biodestructed and thus to have their hazardous nature destroyed. To generalize, however, about all chlorinated hydrocarbons with existing biotechnology is very dangerous since many of them even with low degrees of chlorination can be non-biodegradable or biologically inactive or may be toxic enough that they cannot be destroyed by biological enrichment techniques. Conversely, highly chlorinated hydrocarbons can be readily biodegraded, e.g., pentachlorophenol (Kirsch, 1973).

Table IV-1 illustrates the percent removal of chlorinated hydrocargons reported from the water streams at conventional wastewater treatment facilities (EPA, 1980). It should be noted that removal does not imply destruction but includes loss into air or sludge. It is likely, however, based upon current studies being conducted jointly between the Environmental Protection Agency and Chemical Manufacturers Association that a significant fraction of chlorinated hydrocarbons removed are being biodegraded in these on-site bioreactors (Romano, 1981). Methods to improve biological degradation of chlorinated hydrocarbons should be applied if the material is found to be present in sludge at hazardous and toxic concentrations. This could be through bioaugmentation, system upgrading, or waste segregation.

E. Toxic Material Spills

Toxic chemicals spilled onto land can be treated biologically if there is not an imminent hazard requiring physical removal of the contaminated material to a landfill or treatment facility. The toxic material must also be biodegradable and at concentrations compatible for microbial growth. One company specializing in spill cleanup has been applying biodegradation in innovative ways in response to particular spill cleanup requirements for several years (OH Materials, Findlay OH). This biotechnology has been demonstrated to be available and more cost-effective than other physical/chemical treatment techniques when time and space allow (Dymerski, 1981). Bioaugmentation, or the addition of biological formulations, has also been found to be of major benefit in these applications, and as a future treatment technology for spills, has some merit as a culture can be specifically adapted to the chemical requiring treatment prior to the spill's occurrence. It has been proposed that a bank of stock cultures be developed for rapid biological response to toxic material spill situations (Chakrabarty, 1981).

Table IV-1

REMOVAL* OF CHLORINATED HYDROCARBONS REPORTED FOR AEROBIC SYSTEMS

	Percent Removed	
Chlorinated Hydrocarbon	Activated Sludge**	Aerated Lagoons**
Bis (chloromethyl) ether	83 (1)	
Carbon tetrachloride	99 (1)	
Chloroform	94 (5)	
Chlorobenzene	93 (3)	
p-Chloro-m-cresol	96 (2)	
2,Chlorophenol	92 (1)	
1,2-Dichlorobenzene	88 (5)	96 (1)
1,4-Dichlorobenzene	93 (5)	81 (1)
1,2-Dichloropropane	82 (1)	
Methylene chloride	99 (1)	92 (3)
Pentachlorophenol	96 (5)	71 (1)
1,1,1-Trichloroethane	91 (5)	96 (1)
1,2,4-Trichlorobenzene	97 (5)	
2,4,6-Trichlorophenol	89 (3)	99 (1)
Tetrachloroethylene	98 (5)	
Hexachlorobenzene	94 (2)	
Trichloroethylene	95 (5)	

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* Percent removal is not percent destroyed. Volatilization or adsorption onto or absorption into cells could occur without degradation.

** Number of reported data in parentheses.

Ref: Volume III, Treatability Manual, Technologies for Control/Removal of Pollutants, EPA-600/8-80-042c.

Any one of a number of methods can be applied depending upon the geology of the site, the nature of the spill, and the extent of areal and subsurface contamination. Figure IV-2 illustrates a typical layout for a spill site remedial system used by OH Materials (Walton and Dobbs, 1980).

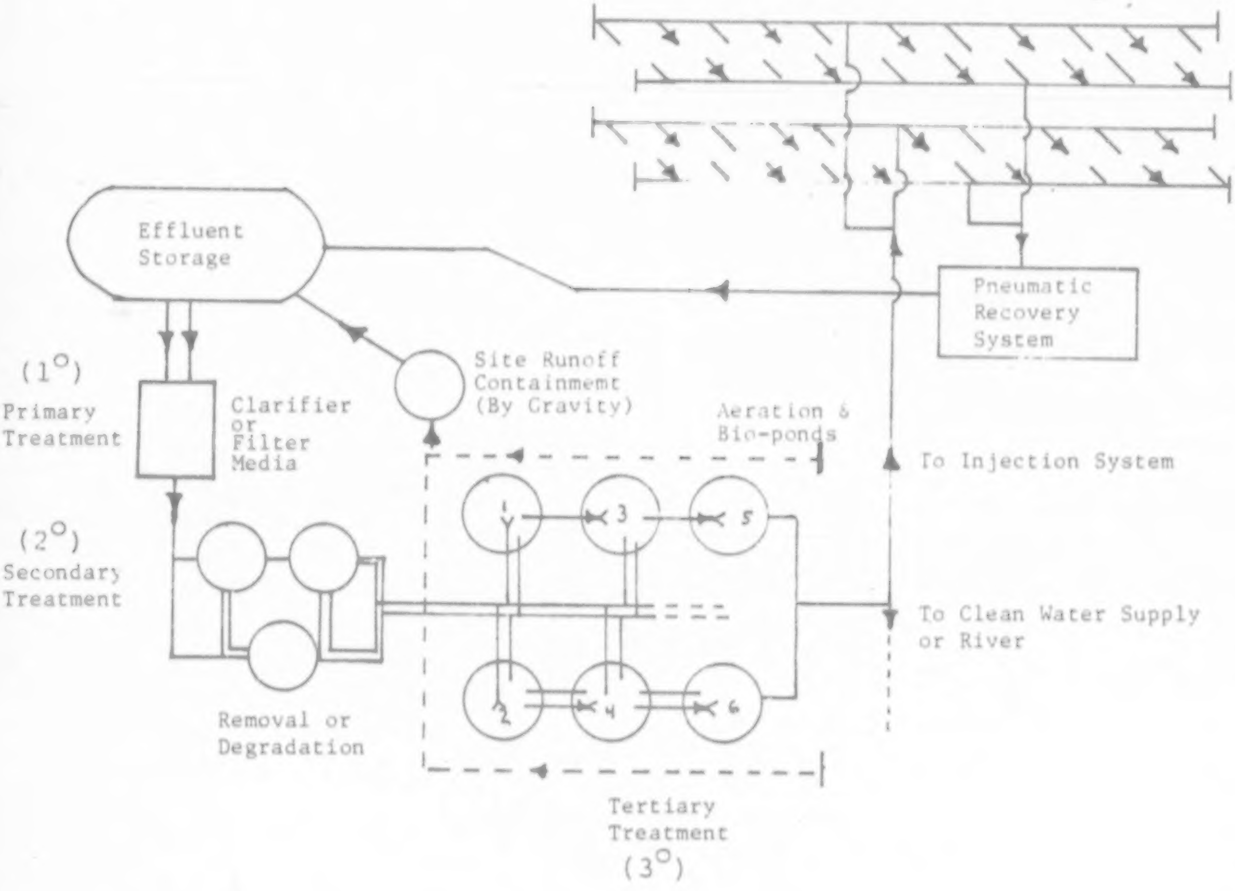

Figure IV-2

TOP VIEW OF TYPICAL PROCESS FLOW CHART FOR SPILL CLEANUP (Walton and Dobbs, 1980)

The diagonal arrows and lines indicate the spill zone where water is injected and through pneumatic recovery contaminated ground water pumped to an effluent storage tank. Depending upon the characteristics of the waste the toxic material carried in the recovered water can be treated physically/chemically or biologically in the 1°, 2°, and 3° treatments. The treated effluent is then either discharged to surface water or returned to the spill zone. In situ biodegradation is accomplished through bioaugmentation by adding a biological formulation to the storage tank and reversing the flow to seed the subsurface zone.

There are two cases where this bacterial treatment system was reported by Walton and Dobbs. For an acrylonitrile incident, after air stripping through the system reduced ground water concentrations to levels tolerable for microbial growth, bioaugmentation with nutrients accomplished a reduction in ground water from greater than 1,000 ppm to less than 0.2 ppm in three months. In the second incident, a similar approach in treating a phenol and o-chlorophenol spill through bioaugmentation resulted in phenol reductions from approximately 30 ppm to less than 0.02 ppm, and o-chlorophenol reductions from over 100 ppm to 40 ppm, in less than one month.

It should be noted that there have been no carefully controlled experiments to verify the results observed; however, the data presented in the above case appears as a reasonable explanation for the loss in concentration, even though the method of analysis reported only one well and the possible convective transport of the contaminant out of the subsurface sampling zone was not noted.

F. Hazardous Waste Remedial Cleanup

Remedial actions at uncontrolled hazardous waste sites are a complex and costly toxic waste problem. Many times sources are not identified until after a health threat exists. Contaminants are usually unknown and sites require significant amounts of investigative work before treatment methods can be developed. Many sites can be classified and treated similar to spills.

During 1980, a survey was conducted to establish the status of remedial measures applied at 169 sites across the country (Neely et al., 1981). Approximately 204 different types of facilities (landfill, dump, drum storage, surface impoundment, injection well, incinerator, and spill) were identified requiring some form of remedial action. The types of cleanup applied were usually containment and/or removal of the hazardous waste. Biological treatments such as land spreading, oxidation ponds, and the enhancement of native microorganisms using fertilizer, were also used but to a lesser extent. In almost all instances complete cleanup was not performed for ground and surface waters as a need to develop more sophisticated technology was required.

The Environmental Protection Agency (Shuckrow et al., 1981) evaluated the use of a combination of six processes (biological, chemical coagulation, carbon adsorption, resin adsorption, membrane processes and stripping) to treat leachates and ground/surface waters contaminated with 12 chemical classes of hazardous waste, ten of which were organic. Of the five process trains examined, biological technologies were involved in all but one, the results indicating the wide potential of biological technologies in treating hazardous wastes at uncontrolled sites.

Various process trains based on the above were evaluated in bench scale studies for the treatment of contaminated ground water at a site in Muskegon, Michigan (James and Shuckrow, 1981). The toxic pollutants identified included vinyl chloride, 1,1-dichloroethylene, 1,1 and 1,2-dichloroethane, benzene, 1,1,2,2 tetrachloroethane, and toluene. Concentrations of these pollutants ranged from non-detectable to 10 percent. It was found that granular activated carbon (GAC) followed by an activated sludge system was successful in removing the toxic pollutants. Anaerobic treatment was also investigated in the form of an upflow anaerobic filter and was found to be able to treat the leachate though not as efficiently as the activated sludge system.

G. Pretreatment Technologies

Several processes, both physical/chemical and biological, may become available within the next several years for on-site or off-site use to render complex, diverse types of substituted organic chemicals into more uniform states readily assimilated by biological treatment systems. Worne Biochemicals, Inc., for example, a major supplier of biological formulation, has recently announced an OXYPHOTON$^{(TM)}$ process for use in dehalogenating and oxidizing complex substituted organics and cyanides into uniform biological precursors (Worne, 1981). The process uses low amounts of energy and, though considered expensive in practice for cyanides, may be quite cost-effective for hazardous organic sludges (Wachinski, 1981). The process is depicted in Figure III-2. Enhanced photodecomposition of halogenated organics is a well known phenomenon occurring with U.V. irradiation (Christensen and Weimer, 1979; Kaufmann, 1981) and may have wide application as a pretreatment process for hazardous waste. Other studies of a more fundamental nature have indicated that anaerobic systems may have a capability to dehalogenate complex substituted aromatic compounds (e.g., methoxy chlor) rendering them more amenable to biooxidation (Fogel, 1981). Such an anaerobic process, in the form of a roughing filter, may have applicability to dehalogenating organic chemical or pesticide wastes. In the event biological technologies combined with pretreatment are developed, economics may justify their use at the source of waste generation rather than transport of waste off-site.

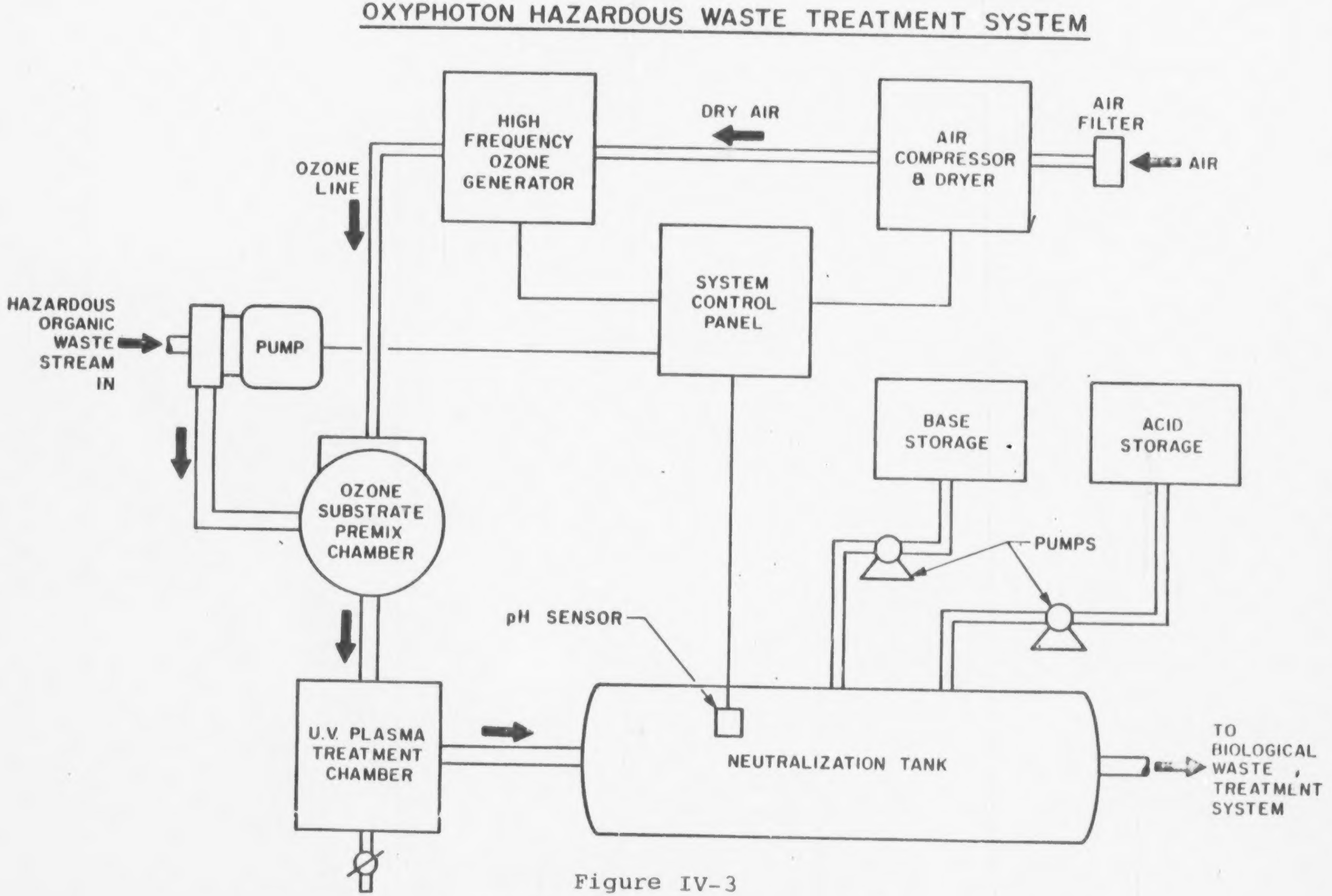
OXYPHOTON HAZARDOUS WASTE TREATMENT SYSTEM
HIGH FREQUENCY OZONE GENERATOR
DRY AIR
AIR COMPRESSOR & DRYER
AIR FILTER
AIR
OZONE LINE
SYSTEM CONTROL PANEL
HAZARDOUS ORGANIC WASTE STREAM IN
PUMP
BASE STORAGE
ACID STORAGE
OZONE SUBSTRATE PREMIX CHAMBER
PUMPS
pH SENSOR
U.V. PLASMA TREATMENT CHAMBER
NEUTRALIZATION TANK
TO BIOLOGICAL WASTE TREATMENT SYSTEM

Figure IV-3

OXYPHOTON PROCESS (Worne, 1981)

Pretreatment may also involve emulsifying insoluble, highly concentrated still bottoms into fine particulate forms with a large surface to mass ratio for subsequent biological or thermal treatment. Petroferm (Misrock, 1982) has been developing a mucopolysaccharide obtained through bacterial fermentation that has a high emulsifying property for such applications.

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V. MARKET ANALYSIS

The biotechnology industry in hazardous or toxic waste management supplies products and services associated with anaerobic, aerobic, and land based treatment methods. In contrast to physical/chemical techniques, biological technology applications share few of the off-site hazardous waste markets* under RCRA. Their major applications are found onsite, primarily in the treatment of toxic waste water streams or the land farming of sludge. The market and its potential growth through biotechnology equipment and the supply of bacterial formulations are presented.

A. Biotechnology in Relation to the Total Hazardous Waste Market

It is difficult to predict what the total cost of compliance will be in the future as a result of hazardous waste controls. EPA estimates of $500 million per year apply to 27 percent of the generators (EPA, 1980) or extrapolated to 100 percent would be nearly $2 billion dollars per year. On the other hand, some chemical industry estimates are as high as $10 billion and others for industry on the whole are as high as $50 billion.

According to the Department of Commerce, Bureau of Economic Analysis, estimates of total annual capital and operating costs for water and solid waste pollution abatement in the manufacturing sector are approximately $12 billion (55 percent water) for 1981 (O'Connor, 1981). This information is published periodically (Rutledge et al., 1981 a,b), and is based upon survey estimates directly from the companies. On the other hand, the Bureau of Census publishes statistics directly from aggregated data supplied annually by industry. Their data for pollution abatement (solid and water) for the manufacturing sector were approximately $6 billion (75 percent water) for 1979 (McCaughey, 1981) or adjusted for inflation approximately $7 billion for 1981. Assuming a reasonable average for these two survey sets we estimate an approximate range for 1981 of $9-11 billion, a subset of which includes hazardous waste costs.

It is difficult to ascertain what portion of these costs for the manufacturing sector are due to the control of toxic pollutants; however, we estimate approximately 40-50 percent hazardous (toxic) and 50-60 percent non-hazardous (non-toxic). This is based on three assumptions: (1) the estimated size of the 1980 solid hazardous waste market (approximately $1-2 billion)(Frost and Sullivan, 1981); (2) a 50 percent allocation of water treatment in the manufacturing sector to oil/grease, toxic substances and others (O'Connor, 1981) (or about $3.3 billion 1981 dollars); and (3) manufacturing industries' estimated annual expenditure to comply with BPT/BAT under the Clean Water Act (Gianessi & Peskin, 1980) (or about $1.5 billion 1973 dollars for petroleum, chemicals, rubber, and primary metals). In 1981 dollars (2) and (3) are approximately the same, or $3 billion.

The following chart provides our estimate of the relation of biotechnology to water and solid waste market expenditures for 1981.

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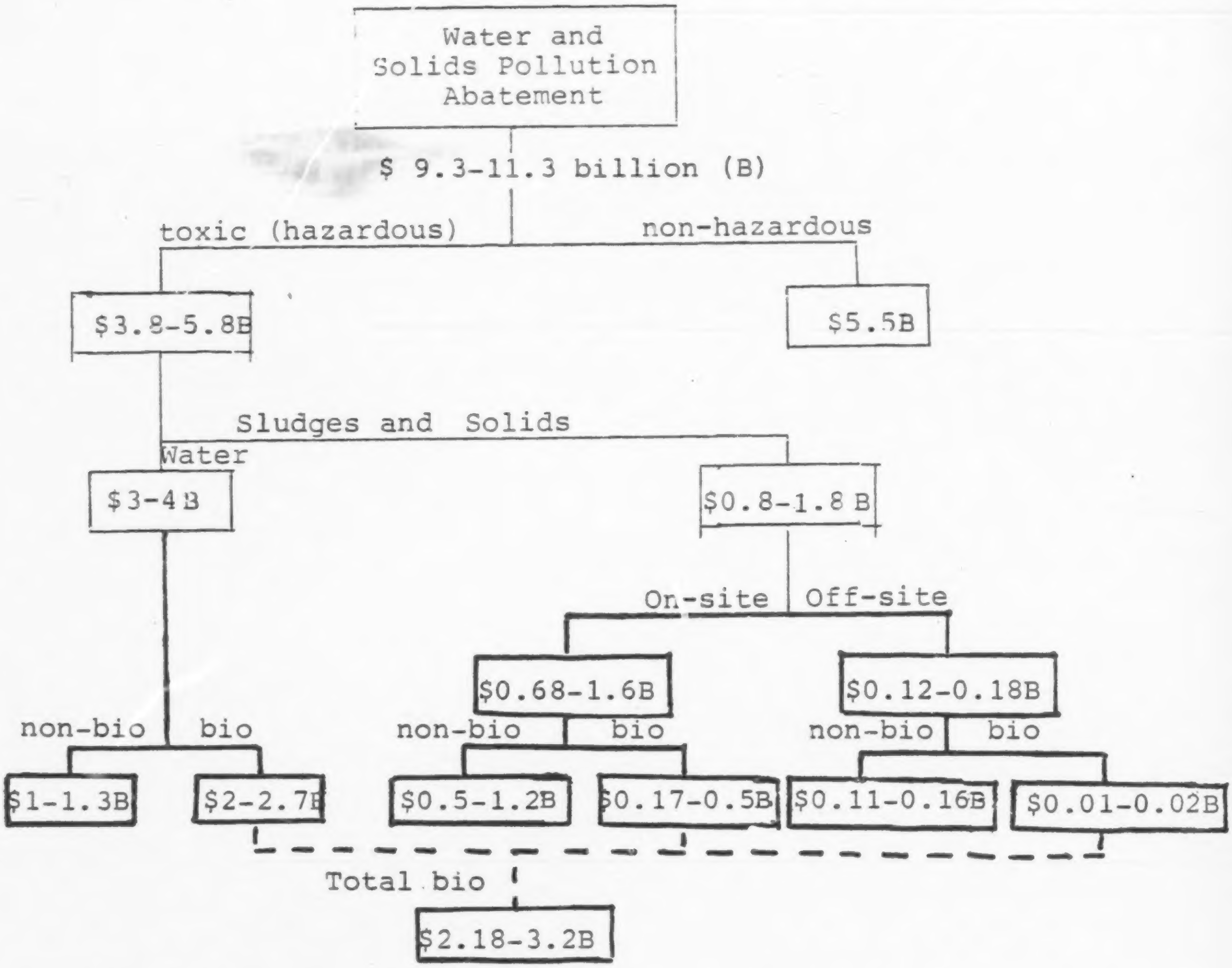
*minor markets will be in cleaning and recovery



(SOURCE:AmTech Cons.)

Figure V-1

ESTIMATED BIOTECHNOLOGY EXPENDITURES FOR 1981
IN TREATING TOXIC WASTES FROM THE MANUFACTURING SECTOR

In the treatment of toxic waste water streams from the manufacturing sector, biological technologies are estimated to represent about two-thirds of treatment methods. Discussions with members of the chemical industry (Schroy, 1981; Campbell, 1981; Romano, 1981) confirm that biological technologies are widely used and quite efficient in treating toxic water discharges for this sector. For solid hazardous or toxic waste, estimated biological treatments (e.g., land farming of sludges) show only 25-30 percent of the market. For off-site treatment, that is, treatment off-site by a non-generator on a fee basis, there is very little application of biological techniques according to the large waste management firms (Chemical Waste Management, 1981; SCA, 1981; IT Corporation, 1981).

B. Market Profile and Potential Market

The biotechnology industry in hazardous waste management can be fairly well divided into three primary categories, with many companies overlapping in the areas:

- Manufacturers and suppliers of equipment and materials
- Consulting engineers and service organizations
- Operators of treatment facilities

Large generators of hazardous waste can provide all categories of services internally within their own companies but frequently seek outside expertise and purchase equipment for their on-site operations. Equipment suppliers operate independently of the waste generators and supply services for both on- and off-site management. There are over 100 firms that supply various types of products in the biotechnology market, most of these transferring their know-how from industrial and municipal waste treatment to hazardous waste. There are also several large integrated companies that provide routine services for hauling and off-site disposal and a wide mix of small firms that have grown in response to toxic spills cleanup and reclamation of uncontrolled hazardous waste sites. Novel hazardous waste treatment approaches using biological methods are frequently used by these firms and are expected to be attracted to the on-site hazardous waste treatment market.

Though the volume of sales is expected to steadily increase for the large integrated transporters and disposers of hazardous wastes, the largest market for biological technologies is expected to be diverse and for use as an on-site treatment technology. There are several factors influencing this direction, primarily the liability associated with passing the health risk to another (ARCO, 1981), capacity limitations for off-site disposal (Frost and Sullivan, 1981) and biological technology applications. However, the difficulty in permitting any new facility under the current hazardous waste regulations may have industry opt for off-site disposal by a contract hauler; however, upgrading existing facilities may be more effective (Mobil, 1981).

The following industries generate organic wastes which could be amenable to biological treatments. (Refer to Table VI-1 for more specific quantities.)

Table V-1

QUANTITY OF HAZARDOUS WASTE GENERATED BY MAJOR SECTOR (EPA, 1980)

Industry	Quantity (MMT/Y)*
Textiles	270
Paint and allied products Organic and synthetic chemicals	28,700
Petroleum refining and coal products	950

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*MMT - Thousand Metric Tons

Permitting of on-site treatment and disposal facilities should be in progress within the next 1-3 years (Shomaker, 1981), and suppliers of services and equipment in biological technologies will benefit through upgrading existing facilities and applying new techniques.

It is extremely difficult due to the lack of accurate data, regulations, and available technologies, to estimate the potential market for biotechnologies in treating hazardous wastes. Many hazardous wastes amenable to biological treatment can be present in wastewater streams or sludges from manufacturing industries and also present in the 7,000 or so surface impoundments identified by the EPA as RCRA facilities (EPA, 1980).

A questionnaire was submitted to approximately 30 industries representing a cross-section of hazardous waste management interests (11 consulting engineering firms, 7 on- and off-site operators, 7 biotechnology companies, 3 trade associations, and 1 regulatory agency). Companies were mostly selected at random from trade journals listing firms performing hazardous waste treatment. They were phoned, a questionnaire sent, and a follow-up phone call made. Of 12 written responses, six estimated the following percent of the pollution abatement market biological technologies will occupy.

	1982	1985	1990
Industrial wastewater			
range	20-90%	35-90%	35-90%
n	5	5	5
Solid waste			
range	30-50%	30-65%	30-80%
n	3	3	3
Uncontrolled sites			
range	10-20%	1-50%	15-60%
n	3	4	3
Spill cleanup			
range	10%	2-25%	20-25%
n	2	3	2

The sample size is probably insufficient and possibly biased towards interests responding that have biotechnology capabilities in hazardous waste control.

A conflict between competing on-site and off-site sectors is probable in the cleaning of existing ponds and lagoons. On the one hand, those deploying biological technologies see this as a significant opportunity to clean "in place" the lagoons using bio-augmentation and aeration practices and eventually retrofitting the facility for on-site treatment (Dymerski, 1981). On the other hand a large waste management firm may prefer using its existing transport capabilities for physically pumping "clean" the lagoons and shipping the waste for centralized treatment or disposal elsewhere (Frost and Sullivan, 1981). The choice is site specific and is a function of the value of land reclaimed and cost of on-site treatment vs. the cost of cleaning, hauling and off-site disposal. To put the size of the potential on-site/off-site market into perspective, of the estimated $1-2 billion dollar hazardous waste market in 1980, approximately 10 percent went to the off-site sector. Including the on-site operations managed by hazardous waste management firms, the major off-site industries account for less than 15 percent of the total market (Frost and Sullivan, 1981).

Many opinions have been expressed over what directions the large companies will swing, towards on- or off-site treatments. Some feel that it will be cheaper for the large generators, such as chemical companies, to let hazardous waste management firms take care of their waste off-site (Frost and Sullivan, 1981). However, as many of these companies have extensive inhouse capabilities on environmental and biological control techniques, it is likely that they will be quick to pick up and implement new on-site treatment processes when confronted with the decision (ARCO, 1981). These choices, however, are all a function of cost-effectiveness but off-site shipment also carries possible liability concerns between the generator and hauler as well as possible resource recovery.

1. Manufacturers and Suppliers of Equipment and Materials

As previously mentioned, the manufacturers and suppliers of equipment and materials that support biological technology applications are quite diverse and are for the most part companies that have developed product lines in the waste water sector and will transfer these goods to hazardous waste biological applications (Envirex, 1981). Table V-2 lists the types of products applicable. This transfer may be more apparent if the multibillion dollar construction grants program is significantly reduced in size forcing some of those supply industries to find new outlets for their goods. This is apparent as two-thirds of the absolute increase in water pollution control equipment expenditures since 1972 ($5.4 billion to 7.1 billion 1972 dollars) have been attributed to the federal construction grants or similar state programs (Rutledge, 1981b).

Table V-2

TYPES OF PRODUCTS IN THE BIOLOGICAL TREATMENT OF HAZARDOUS WASTES (JWPCF, 1981)

Aerators
Analytical Testing
Bacterial Cultures
Bioassays
Centrifuging
Chemical Feed Equipment
Chlorination Equipment
Clarifier Equipment
Coagulants
Coatings and Linings
Computers
Controls
Conveyers
Corrosion Protection
Digestion Tank Equipment
Disinfection, Ultraviolet
Dissolved Oxygen Analysis Equipment
Distributors, Rotary
Drives, Variable Speed
Dryers
Engines (Sludge gas)
Filter Equipment (misc.)
Flocculants and Equipment
Flotation Equipment
Flow Measurement
Gas Control and Holders, Boilers
Gas Engines
Gaskets
Gates
Generators
Gratings
Grease Control
Grinders
Grit Collection
Heating Equipment (Digesters)
Irrigation Systems
Joints, Compression and Mechanical
Lift Stations
Liners (Ponds and Reservoirs)
Lubricants
Manhole Equipment
Masonary Materials
Meters
Mixing Devices
Monitoring Devices
Motors
Nozzles, Spray
Odor Control Systems
Oxygen Activated Sludge
Ozone Generators
Pipe
Presses
Pumps, including Sludge
Rotating Biological Contactors
Safety Equipment
Sampling Equipment
Screening Equipment
Sealants
Shredders
Siphons
Sludge Systems
Switch Gears
Tank Covers
Tanks
Transformers
Turbines
Valves
Vapor Absorbers
Water Separators
Weighing Devices

Companies that supply products for advanced waste treatment, digestion tank equipment, oxygen activated sludge, and irrigation systems, are listed in Table A-1 of the Appendix.

A general estimate of equipment sales in biological waste treatment can be made on an aggregated basis considering statistics on expenditures for the following manufacturing sectors producing non-durable goods (Rutledge et al., 1981b).

Table V-3

ESTIMATE OF 1981 CAPITAL EXPENDITURES FOR
BIOTECHNOLOGIES IN MANUFACTURING SECTOR
(Rutledge et al., 1981b)

	1981 ($million)		f*		1981 Est. ($million) Biotechnology		
Manufacturing Sector	Water	Solid	W	S	Water	Solid	Total
Textiles	30	5	.5	.4	15	2	
Chemicals	340	110	.8	.3	272	33	
Petroleum	910	180	.9	.2	819	36	
Rubber	20	10	.9	.3	18	3	
Primary Metals	280	70	.3	.1	84	7	
					1,208	+ 82	1,290

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f* - fraction of expenditure estimated for biological treatment of wastes. Based upon effluent guideline documents for water and RCRA background documents (EPA library and public document room, 401 M Street, S.W., Washington, D.C.).

Because of the previously cited differences in cost data results and the fact that other industries are not included above, e.g., coke and steel, industrial solvent dischargers and other minor manufactures, these estimates could be high or low. Assuming this high-low range is 25 percent** around the $1.290 billion total above, we estimate that in 1981 biotechnologies probably accounted for product purchases of 1 to 1.6 billion dollars for the control of hazardous/toxic pollutants in water and solid waste streams, mostly onsite.

Of particular interest are companies involved in the production of biological formulations or engaged in research and development in genetic engineering that will provide for new opportunities in the biological treatment of hazardous wastes. A major manufacturer of advanced waste treatment systems, Envirex, Inc. (Martin, 1981), feels that this area will contribute greatly to new methods of biological treatment of hazardous waste.

**25 percent is the approximate variation around the mean of Rutledge (1981a,b) and McCaughey (1981) data presented in the previous Section V.A.

There are many companies involved in the production or research and development of bacterial formulations for hazardous pollution abatement. Some of these are quite small operations and are not major suppliers (Polybac, 1981) and others are engaged primarily in contract research. The largest in the field are Sybron Corporation, Cytox/Polybac, and Flow Laboratories. The following companies have been referred to from various sources, with total sales from $4-5 million dollars in 1980 for biological formulations sold to industry (First, 1981; Glick, 1981; JWPCF, 1981; Hyde, 1981).

Projection of Product Sales

Hazardous waste management will provide for product sales in biological treatments of toxic solid and liquid wastes, the cleanup of spills on land, and the decontamination of existing uncontrolled hazardous waste sites. The major product sales are expected onsite in the retrofit of lagoons or surface impoundments, upgrading existing wastewater facilities to treat toxic wastes, and the installation of new types of treatment systems to handle more concentrated toxic materials. The use of pretreatment technologies should also provide for increased application of biological methods as a secondary process. It is extremely difficult to forecast future market sizes, and most attempts are speculative; however, the following presents our estimate based upon the data available and potential applications.

There are approximately 7,000 surface impoundments identified by the EPA as RCRA facilities. In a draft economic impact analysis of its interim status standards under RCRA (EPA, 1980), it was estimated by EPA that nearly 50 percent of industry's cost to comply with the interim regulations concerned surface impoundments(EPA, 1980). Total annual costs were $510 million for industries representing 27 percent of waste dischargers. From the data provided, it is estimated that the cost of compliance averages approximately $100,000 per year for each surface impoundment (Table A-2, Appendix). The majority of this cost is for closure/post-closure requirements where waste is removed and the site monitored as a landfill.

It is likely that many of these sites can be retrofitted to biological waste treatment systems (OH Materials, 1981). Sybron Corporation (Telepchak, 1981) estimates that about 20 percent of potential markets can be captured by biotechnologies. Thus assuming 10-30 percent of surface impoundments can be upgraded to biological treatment lagoons at a minimum annual cost of $100,000 and a maximum annual cost of $500,000, the potential market for biological technologies in retrofitting lagoons ranges from a low of $70 million to a high of $1.1 billion per year. A reasonable mean is $600 million per year within the next ten years.*

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*Many lagoons may have recoverable materials which could be facilitated through the use of biological methods.

Table V- 4

COMPANIES ENGAGED IN SALES OF BIOLOGICAL FORMULATIONS OR GENETIC ENGINEERING FOR HAZARDOUS WASTE

Company	Total Sales	Estimated Sales to Industry	Export	Research & Development
Biotech, Incorporated	< 500,000	0		Heavy metals (contract research)
Biotechnica International	none			Thermophile interests
Cytox/Polybac	1,200,000	1,140,000		Toxics degrading, spills
Flow Laboratories	1,500,000	750,000		New formulations
General Environmental Science Corporation	500,000	400,000		
Microlife Technics	1,500,000	600,000		Genetic Engineering (Petrochem Waste)
Petroferm	NA			Oil spills and cleaning
Reliance Chemicals	250,000	125,000		
Sybron Corporation	1,500,000	1,425,000		PCBs, chlorinated hydrocarbons
Worne Biochemicals	450,000	225,000		Pretreatment processes
	$7,400,000	$4,665,000		

NA - Not available

(SOURCE: AmTech Consultants)

Direct on-site treatment of specific waste streams are also major candidates for biological treatment technologies. Of the approximately 50,000 MMT targeted under RCRA-C (Final Environmental Impact Statement, 1980), 20 percent might be treated biologically. (Of wastes already listed, Table VI-1 identifies a potential 1,000 MMT.) With a biological treatment cost of 0.02 to 0.3 dollars per pound (within ranges indicated in Figure III-4), $450 million to $6,600 million is the potential market size by simple multiplication. The likely market for capital expenditure is probably within the $1.5-3.0 billion range by 1990 for on-site treatment using biotechnology with or without pretreatment.

Off-site use of biological systems will be minimal as most biotreatable waste will be handled by the generator and not sent off-site.* The maximum market is estimated to be 10 percent of the off-site waste management industry. This would be $63-163 million using the Frost and Sullivan off-site hazardous waste management market size for 1990 (Frost and Sullivan, 1981).

Table V-5 presents our estimated growth of the hazardous waste biotechnology product market over the next eight years. Overall the growth of the market is expected to be the greatest for biological controls in the RCRA waste areas. Near-term growth (1-4 years) in RCRA-related areas is expected in the retrofit and modifications of lagoons or surface impoundments as well as development of on-site biological treatment technologies. These on-site biological treatment technologies can be the upgrading of industrial wastewater plants to treat RCRA-type wastes, as well as the use of pretreatment technologies.

Our key assumptions in making this forecast are that

- the total hazardous waste market will have an annual growth rate of 25 percent in the next decade to $3-8 billion (Frost & Sullivan, 1981);
- biotechnologies, which outside of industrial wastewater treatment facilities currently handle little hazardous or toxic waste, will outstrip the growth rate of the total hazardous waste market in the near term by:
    - upgrading existing lagoons
    - upgrading conventional wastewater treatment facilities to handle more toxic waste;

  thus, an overall annual growth rate of 40 percent is estimated in 1982-1986;

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*Dr. Irving Solomon, Director of Research for Chemical Waste Treatment, Inc., in phone discussion stated that biotechnology would probably not be applicable offsite in the future. If waste was biologically compatible, it would most likely be handled onsite, leaving the more refractory materials to offsite disposers.

Table V-5

ESTIMATED PLANT EQUIPMENT MARKET POTENTIAL FOR BIOTECHNOLOGY IN HAZARDOUS WASTE TREATMENT ($MILLION 1980)

Pollution Abatement Sector	1982	Annual Rate	1986	Annual Rate	1990
"RCRA" hazardous waste					
lagoon retrofit	50-90		200-400		500-1000
on-site treatment*	300-600		1000-2000		1800-3800
off-site treatment	5-15		35-165		80-350
	355-715	40+%	1235-2565	20+%	2380-5150
Industrial wastewater (with toxic waste)	750-1300	10%	1200-2200	10%	2000-3500
Spill cleanup	30-50	55%	150-300	10%	150-350
Uncontrolled sites	20-50	5%	30-60	45%	150-250
DOMESTIC TOTAL	1155-2115		2615-5125		4680-9250
Export activities					
solid	60-100		70-140		100-400
water	50-75		50-110		80-380
spill	10-15		20-50		25-100
uncontrolled	1-5		5-10		10-30
	121-195		145-310		215-910
TOTAL	1276-2310		2750-5435		4895-10160

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*Includes a transfer of 30 percent industrial waste water treatment to RCRA generator compliance sector

(SOURCE: AmTech Consultants)

- following this near-term upgrading period, the proximate annual growth rate for the total hazardous waste market will be followed (20 percent/year) as pretreatment, innovative cell lines, and fermentation techniques become available to keep pace;
- the potential market sizes previously discussed for upgrading lagoons ($600 million/yr) and directly treating discharges ($2-4 billion) will be met by 1990.

A sample calculation is provided in Table A-3 (Appendix).

Many developments in the RCRA area are directly related to on-site industrial wastewater treatment, and the growth in this latter market is expected at 10 percent per year, following the recent trend for water expenditures for the manufacturing sector (Rutledge et al., 1981b), except that a 30 percent fraction has been transferred to the RCRA category starting the 1982 base year as a considerable number of wastewater plants are listed as generators of toxic waste (sludge) (see Chapter VI and EPA Hazardous Waste Listing).

As discussed with manufacturers of biological formulations (Polybac, 1981; Sybron Corporation, 1981) and spill cleanup companies (OH Materials) it is estimated that spills will receive considerable attention in the near-term for the use of biological techniques with the applications switching to uncontrolled hazardous waste sites in the mid-term. A growth in this sector does not imply that new uncontrolled sites will be discovered but that biotechnologies will be developed in response to existing remedial action needs at these sites, which can take 5-10 years to clean up, the final steps of which include low-level decontamination compatible with biological manipulation techniques such as in situ bioaugmentation.

Bioaugmentation will play an increasingly significant role in the treatment of hazardous/toxic waste. Dosages are fairly consistent between products and approximate 2 kg per day per 50 l/s of flow but vary depending upon the waste type. The range of price for various formulations in 1978 was $2-10 per kg. One kg, when mixed with ten liters of water, will contain 10^{10} to 10^{11} microbes per liter. On a per pound organic destroyed basis, this could approximate $0.001-0.006 per lb. depending on maintenance dosage. Bioaugmentation could cost from a fraction of a percent up to 3-4 percent of total treatment costs. (See Figure III-3 to make contrast.)

Table V-6 presents our estimated market forecast for biological formulations. The high potential is based upon the maximum amount of biotechnology application in that sector with intensive bioaugmentation. The low potential assumes minimum biotechnology application and minimum dosages. The likely mean assumes only 20 percent of waste streams will receive a medium dosage as used in sales estimates by Sybron Corporation (1981). The general trend follows sales projection estimates for biotechnology equipment expenditures for each of the main sectors as listed in Table V-5. The results indicate that, though a half-billion dollar potential market will probably exist by the end of the decade, the likely sales will approximate $60 million or an order of magnitude expansion over the current volume.

2. Architectural and Consulting Engineers

Architectural and consulting engineers play a major role in the transfer of technology from the manufacturing sector to the commercial user. They are almost exclusively service organizations though they will often specialize in a particular product line through an affiliated subsidiary or perform waste handling for a client. Due to the demands of the hazardous waste market, new product development and waste management operations are becoming more attractive areas for these firms. These companies can hold or exchange licenses on new technologies and often consult to the government on the development of regulatory criteria.

They also provide regulatory compliance services to industrial clients as well as develop the site specific design and provide operating procedures for new technologies. Many times a manufacturing company may have developed its own technology in-house and in turn provides a consulting service in licensing its use to other interested users (Celanese, 1981).

As a consulting or design engineering service, this sector of the hazardous waste industry usually receives 5-20 percent of the equipment market for its services, depending upon the technology, its availability, complexity, and risk (Etzel, 1981). The following presents our estimate of the size of the consulting engineering market for hazardous/toxic waste treatment using biological technologies (10 percent of value on Table V-5).

Table V-7

ESTIMATED SALES FOR CONSULTING ENGINEERS IN BIOTECHNOLOGY

	(millions 1980 dollars)		
	1982	1986	1990
"Solid" hazardous waste	35-72	144-296	288-615
Industrial waste water	75-130	120-220	200-350
Spill cleanup	3-5	15-30	15-35
Uncontrolled site cleanup	2-5	3-6	15-25
Export activities	12-19	14-31	22-91
	127-231	296-583	540-1116

(SOURCE: AmTech Consultants)

Table V-6

MARKET POTENTIAL OF BIOLOGICAL FORMULATIONS
FOR HAZARDOUS WASTES TREATMENT
(millions 1980 dollars)

Hazardous Waste Sector	1982 A	1982 B	1982 C	1986 A	1986 B	1986 C	1990 A	1990 B	1990 C
RCRA Wastes	1.8	3.8	28.0	7.2	15.5	118.6	14.4	31.7	242
Industrial Waste water	3.8	6.8	52.0	6.0	11.6	88.0	10.0	18.5	140
Spills	0.2	0.3	2.0	0.8	1.6	12.0	1.0	2.3	18
Uncontrolled	0.1	.2	2.0	0.2	0.3	2.4	0.8	1.1	10
Total Domestic	5.9	11.1	84.0	14.2	29.0	221.0	26.2	53.6	410
Exports	0.7	1.1	16.0	0.8	2	30.0	1.5	6	100
Total	6.6	12.2	100.0	15.0	31.0	251.0	27.7	59.6	510

A - low potential
B - likely mean
C - high potential

(SOURCE: AmTech Consultants)

There are numerous consulting engineering firms in this sector ranging from small specialty companies to groups within large architectural engineering firms.

3. Operating Companies

The companies that perform the actual treatment or disposal of hazardous wastes are the purchasers of the above equipment supplies and consulting services. For the most part they are the generators of hazardous waste and perform treatment onsite or at their own off-site facility. They are also independent off-site operators who usually have an established hauling structure to transport wastes under contract to their facility or are companies that respond to spills or the cleanup of abandoned sites. In summary, the "demand" side of the hazardous waste industry is made up of the generators, a half-dozen major off-site operators, and several hundred minor operators.

Biological technologies are most appropriately applied at the source of hazardous waste generation, or onsite, due to the consistent quality and quantity of the waste which is usually required for biological treatment.

As discussed in part one of this section, it is estimated that current biotechnology applications on- and off-site have $1-1.6 billion in capital costs with the sizable majority being treatment of toxic waste in industrial wastewater streams, and land application of sludges.

Because of the early stage of development of the RCRA Subtitle C regulations its orientation is towards traditional solid waste generation and control of off-site disposal. The overall market focus has largely been on services associated with off-site waste hauling, storage, treatment and siting; and related engineering and legal expertise for design of physical chemical processes and regulatory compliance (such as manifests, permits, financial requirements, etc.). This off-site industry is dominated by approximately a half-dozen waste management firms that specialize in transport and off-site disposal.

Table V- 8

MAJOR HAZARDOUS WASTE MANAGEMENT FIRMS
(Frost & Sullivan, 1981)

Company	1980 Sales (millions $) Total	Hazardous Waste
Waste Management, Inc.	500 - 550	100
Rollins Environmental Services	400 - 425	40
Browning Ferris Industries	560 - 600	40
SCA Services	250 - 275	30
Cecos International	75	30
IT Corporation	45	5
Chem-Nuclear	40	2

Because biological technologies require a consistent quality and quantity of waste for successful treatment, most of the above firms focus their off-site treatments on physical/chemical methods which can destroy variable types of hazardous materials received. However, most of these firms use traditional biooxidation processes to treat their waste water discharge streams at off-site treatment facilities. These firms are actively looking at primary biological processes but their use is precluded by the variable nature of the waste they receive (Chemical Waste Treatment, 1981).

On-site disposal of hazardous waste is expected to provide the greatest market for biological methods due to the relatively consistent nature of the waste at the source of generation. There are over 65,000 generators of hazardous waste that have notified EPA (Tamarken, 1981), and an estimated 25,000 - 30,000 hazardous waste treatment facilities in the country (EPA, 1980)*. The following represents the mix of types of operations at these facilities.

Surface impoundments	26%
Land disposal and treatment	17%
Incinerators	6%
Storage facilities	51%

Aside from conventional wastewater treatment, a major method of biological treatment of hazardous waste by generators is through land farming. A recent survey by EPA (Wyles, 1981) indicated that of 151 sites ranging from 0.1 to 1,600 acres, over 90 percent were operated by generators.

Projection of Operating Costs

Operating costs of biotechnologies are expected to follow the same trend as for the sale of products (capital expenditures) in Table V-5. Their high operating to capital ratio (4.5:1) for solid waste (O'Connor, 1981) is probably due to expenditures for off-site contract delivery, and the low capital requirement for existing landfills or other solid waste disposal techniques. Land farming, for example, is more operating than capital intensive. Assuming the ratio for biotechnology application to solid waste as 2:1 for the present, the following is the combined annual estimate of expenditures for 1981.

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*Since the publication of this report in April 1980, based upon preliminary notification of hazardous waste activity on 2/26/80 (Sec. 3010 RCRA), 14,000 permit applications have been received (Sec. 3005 RCRA).

Table V-9

ESTIMATED CURRENT ANNUAL COSTS (1981) OF BIOTECHNOLOGY APPLICATIONS IN HAZARDOUS/TOXIC MATERIALS CONTROL ($MILLIONS)

	Water	Solid	Total
Capital	1208	82	1290
Operating	1400	164	1564
Total	2608	246	2854

(SOURCE: AmTech Consultants)

Future biotechnology operating costs are probably more in line with wastewater treatment ratios of 1:1 as more capital equipment, as described in Table V-5, is installed. Capital investment occurs at one time and is financed over the lifetime or less, of the technology. The data in the table shows 1981 capital expenditures for new technology and 1981 costs for operating those that already exist. It will require operating costs to run the biotechnology plant expenditures forecasted in Table V-5 at an assumed 1:1 ratio according to past trends in wastewater (Rutledge, 1981a). Therefore, the potential operating costs for 1982, 1986, and 1990 are $1.1-2 billion, $2.5-5.0 billion, and $4.5-10 billion, respectively.

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VI. BIOTECHNOLOGY AS A MAJOR HAZARDOUS WASTE TREATMENT TECHNOLOGY - FACTORS AFFECTING DEVELOPMENT

There are two major areas that are important to the future development of biotechnologies for treating hazardous waste. First, there are the types of wastes considered as hazardous or toxic that require treatment and the public and private research activities that are essential in developing new and innovative technology approaches. Second, there are the forms of legislations and regulations that affect research appropriations and market responses and the overall institutional barriers that may confront the development. In this chapter we present these two major areas plus a mix of brief scenarios on the possible market responses.

A. COMMERCIAL APPLICATION AND RESEARCH AND DEVELOPMENT POTENTIAL

1. Potential for Biotechnology as a Primary Treatment Method

Biotechnologies are currently used to treat a wide variety of hazardous wastes. However, these applications are most frequently found when toxic materials are co-disposed and treated as conventional pollutants in wastewater treatment facilities or in traditional land application of sludges containing hazardous/toxic materials. Many toxic materials classified as hazardous are more persistent than conventional non-toxic pollutants and resist biodegradation in the normal operation of conventional biological treatment processes. Consequently, biological treatment of the more persistent toxic wastes requires either a physical/chemical pretreatment to render them more amenable to biodegradation, an alteration of system design to allow for lower concentrations or longer contact between the biota and the toxic chemical, or manipulation of the biology of the system to allow for an increased presence of the enzymes responsible for catalyzing the destruction of the toxic material.

The objective in waste management is to recover material to the maximum extent economically feasible and, if discharged into the environment, to render the material detoxified, immobilized, or completely destroyed. Hazardous toxic wastes have primarily been managed through co-disposal in conventional biological treatment systems, destroyed through thermal processes such as thermal oxidation in rotary kilns and incinerators, chemically treated through alkaline/acid hydrolysis, injected in deep wells, or landfilled or disposed. Conventional biological treatment processes are used for wastewater streams containing dilute concentrations of toxic organic materials and the sludges generated in such processes. These water and sludge streams are considered a hazardous waste if they contain toxic concentrations of the waste.

Table VI-1 presents information we developed from the quantity and quality of non-specific and specific sources of hazardous waste

Table VI-1

POTENTIAL APPLICATIONS OF BIOTECHNOLOGY FOR HAZARDOUS WASTE TREATMENT *

Industry	Number	Basis for Listing	Form	Quantity** (MT/Yr)	Current Disposal Practice	Biological Method	Potential Probability
General (degreasing)	F001	tetrachloroethylene methylene chloride tri chloroethylene 1,1,1 trichloroethane chlorinated fluorocarbons carbon tetrachloride	liquid (conc) sludges	99,000	landfill incinerated deep wells	dilution anaerobic	med/low
General (solvent recovery)	F002 F003 F004 F005	same cresols cresylic acid nitrobenzene others	same still bottoms	100-200,000	same	same/ aerobic	med/low
Organic Chemicals							
acetaldehyde	k009 k010	chloroform formaldehyde methylene chloride methyl chloride paraldehyde formic acid	waste-water	590,000 @ 5% conc.	injection	anaerobic	med/high
acrylonitrite	k011 k012 k013 k014	acrylonitrile acetonitrile hydrocyanic acid acrolein acrylamide	waste-water	2-3,000,000 @ 8% solids (inorganics)	ponding injection alk. hydrolysis	pretreat/ aerobic	med/low
benzyl chloride	k015	benzylchloride chlorobenzene toluene benzotrichloride	still bottoms (sludges)	280	incinerate landfill	dilution/ aerobic	med
carbon tetrachloride	k016	hexachlorobenzene hexachlorobutadiene carbon tetrachloride hexachloroethane perchloroethylene	still bottoms	3,200	landfill incinerated	dilution/ aerobic/ anaerobic	med/low

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**MT - metric ton

(SOURCE: AmTech Consultants)

Table VI-1 (Continued)

Industry	Number	Basis for Listing	Form	Quantity (MT/Yr)	Disposal Practice	Biological Potential	
						Method	Probability
epichlorohydrin	k017	epichlorohydrin chloroethers dichloropropane dichloropropanoals	still bottoms	10,000	incineration	dilution/ anaerobic	med
ethyl chloride	k018	1,2 dichloroethane trichloroethylene hexachlorobutadilene hexachlorobenzene	sludges heavy ends	35,000	land disposal incineration	dilution/ aerobic/ anaerobic	med
ethylene-dichloride vinyl chloride	k019 k020	ethylene dichloride 1,1,1 trichloroethane 1,1,2 trichloroethane tetrachloroethanes chloroethylenes carbon tetrachloride chloroform vinylchloride vinylidenchloride	sludges heavy ends	>120,000	landfill incineration	dilution/ anaerobic	med
phenol/acetone	k022	phenol tars	sludges	65,000	incineration landfill	pretreat/ aerobic /an	med/low
phthalic anhydride	k023 k024	phthalic anhydride naphthoquinone tars	sludges light heavy ends	7,000	incineration landfill	pretreat/ aerobic	low
nitrobenzene	k025	meta-dinitrobenzene 2,4 dinitrotoluene	sludges	175	landfill	dilute/ aerobic	med
methyl ethyl -pyridines	k026	paraldehyde pyridines picolines	liquid	1,000	wastewater lagoons	dilute/ aerobic	high
toluene disocyarate	k027	toluene disocyanate toluene 2,4 diamine tars	sludges	6,000	landfill	pretreat/ aerobic	low

Table VI-1 (Continued)

Industry	Number	Basis for Listing	Form	Quantity (MT/Yr)	Disposal Practice	Biological Potential Method	Biological Potential Probability
1,1,1-trichloroethane	k028 k029	dichloroethanes trichloroethanes vinyl chloride vinylidene chloride chloroform	liquids/ sludges	10,000	landfill incineration	dilute/ anaerobic	med
trichloroethylene	k030	hexachlorobenzene hexachlorobutadiene hexachloromethane tetrachloroethane ethylene dichloride	liquid sludges	15,000	incinerate landfill injection	dilute/ aerobic/ anaerobic	med
Pesticides							
chlordane	k032 k033 k034	hexachlorocyclopentadiene	wastewater wastewater sludge filtrates		ponding landfills injection	aerobic	med
creosote	k035	creosote polycylics	sludges		ponding	aerobic/ anaerobic	med
disulfoton	k036 k037	toluene phosphorodithiorc acids and esters	wastewater sludges bottoms	200	landfill lagoon/ ponding	aerobic/ anaerobic	med
phorate	k038 k039 k040	phorate formaldehyde phosphorodithiane acid and esters	wastewater sludges filterate		lagoon/ ponding landfill incinerate	aerobic/ anaerobic	med
toxaphene	k041	toxaphene	wastewater sludge	>2,000	landfill ponding	aerobic/ anaerobic	med
2,4,5-T	k042	hexachlorobenze 0-dichlorobenzene	heavy ends		landfill	dilute/ aerobic	med
2,4-D	k043	dichlorophenols trichlorophenols	wastewater sludge		lagoons landfills	aerobic	med

Table VI-1 (Continued)

						Biological Potential	
Industry	Number	Basis for Listing	Form	Quantity (MT/Yr)	Disposal Practice	Method	Probability
Petroleum	k048 k049 k050 k051 k052	chromium lead	sludge	66,000	land farming	aerobic/ anaerobic	med/high
Steel and Iron	k060	cyanide phenolics naphthalene arsenic	ammonia lime sludge	1,000,000	landfill	pretreat/ aerobic	med/low
Chlorine	k073	carbon tetrachloride chloroform dichloroethanes trichlorcethanes tetrachloraethanes	liquid (conc.)	125	incineration injection	dilute/ anaerobic	med
nitrobenzene/ aniline	k083 k103 k104	aniline benzene diphenylamine nitrobenzene phenalenediamine	wastewater streams	160	lagoons landfills	aerobic	med/high
chlorobenzenes	k085 k105	di-, tri-, tetra-, penta-, and hexachlorobenzenes	sludges waste waters	10,000	landfills sand filter/ carbon	pretreat/ aerobic	med med

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*This table was constructed from the RCRA Subtitle C Background Documents, the Appendix VII to 40CFR261.31 and 261.32 and our judgement of its biological potential based upon the waste characteristics as reviewed in Chapter II and potential of biological manipulation techniques. Numbers not presented in series from F001-016 or K001-069 are not in table either because, a background document was unavailable, the waste may have been delisted by the Environmental Protection Agency, or it precluded biodegradation. Some wastes may also be in table that are currently under petition or may have already been delisted. Those reviewed and not presented are F005-F016(CN based could be degraded with carbon source), K002-008-inorganic pigments, K021-Sb catalysts; K031 As, K044-K047 explosives, K061-K063-iron and steel, K064-K069 primary copper, lead, zinc, secondary lead, K071,K076-Cl production, K084, K101,K102-organo-arsenic compounds, K086-inks, K087-coking. Those not reviewed: K001-wood preserving, K053-K059 leather tanning as document was unavailable at EPA.

(SOURCE: AmTech Consultants)

listed by the Environmental Protection Agency and obtained from the background documents (EPA, 1981). The table lists those wastes containing toxic organic constituents as a basis for identification by the Environmental Protection Agency. Though the table presents several million tons of toxic waste, the Environmental Protection Agency plans to list more specific and non specific sources as a determination is made of the criteria and hazard potential. Many other sources, though not specifically listed, can be considered hazardous waste if they meet the criteria established by the Environmental Protection Agency and are therefore subject to control. Also, many of the wastes originally listed by the Environmental Protection Agency have been delisted through petition by the industry. These were mostly wastes containing metals.

A majority of the wastes remaining listed are presented in the table and are generated by the organic chemicals and pesticides industry. The principal types listed are concentrated organic solids or liquid forms that arise from heavy/light ends or still bottoms in the manufacturing processes. They frequently contain traces of the products but usually are unwanted by-products. These are usually landfilled, incinerated, or, if in a liquid form, injected. Also presented in the table are waste water streams containing toxic materials resulting from process water discharge and the sludge from their treatment. These are usually held in lagoons or ponds for treatment, the sludges collected for landfill or incineration, and the water often injected in deep aquifers.

Most of the organics presented in the table have been demonstrated to be biodegraded, thus the potential exists for their treatment through some form of biological process. We have indicated this potential in the right-hand column.

a. Concentrated Organic Solids and Liquids

These materials, consisting of highly concentrated organic compounds, are currently either containerized and disposed of in landfills, or incinerated. Landfills pose a long-term containment risk and incineration can be quite difficult if waste is highly chlorinated or has a high percentage of water. Organic halogen concentrations exceeding 10 percent (for example, trichloroethane contains 80 percent Cl by weight) tend to snuff out the incinerator flame, and other organic materials, primarily fuel oils, are required to dilute the waste stream, thus increasing incineration costs (Wukasch, 1981).

Alternatively, this material could be diluted with water to levels that are compatible for biological treatments either aerobically or anaerobically, and in a dissolved or slurry (sludge) form depending on the physical property of the waste (soluble or insoluble).

Let us take, for example, waste K019/K020 from the manufacture of ethylene dichloride/vinyl chloride. It is estimated by EPA (Background Documents) that over 120,000 MT of this waste is generated per year and is primarily disposed of through landfill, though some incineration is reported. It is primarily a heavy concentrated still bottom with the following estimated composition of chlorinated hydrocarbons.

	Percent
Ethylene Dichloride	25%
Trichoroethane	33%
Tetrachoroethane	17%
Heavy Chlorinated Compounds (Tars)	25%

If incinerated, the waste, containing 70-80 percent Cl by weight, would require a 7:1 dilution with fuel oil for proper flame performance, and the cost of incineration is probably near \$0.3-0.4/lb., with a total disposal cost of \$80 million/yr for the industry.

If landfilled, the material, being hazardous, would require disposal in a secured landfill, with disposal costs estimated at \$0.15/lb. (SCS, 1980). Total annual costs for landfill disposal therefore approximate \$40 million/yr.

Alternatively, let us assume we were able to develop a microorganism capable of mesophilically or thermophilically fermenting this material (dehalogenating anaerobically) in the presence of another carbon source, each at 5,000 mg/l (total of 10,000 mg/l) in a highly mixed enclosed reactor system. Cost: (1) At 5,000 mg/l, dilution 200x would be required resulting in 24,000,000 MT of water, or 6.34×10^9 gallons per year. Water cost (\$0.5/1,000 gals) would be \$3.17 million /yr. (2) Carbon source and nutrients, say molasses, at an equal level of the waste, is also required. At \$0.08/kg, the cost would be \$9.6 million/yr for carbon and nutrient source. (3) A high rate mixed fermenter requiring \$6 per thousand gallons (SCS, 1980) of throughput would cost \$38 million/yr. (4) Assume sludge of 12,000 MT/yr (10 percent cell yield) requiring RCRA disposal at \$6,600,000 (though 99 percent waste is destroyed). (5) Trickling filter for secondary cleanup at \$2.20 per thousand gallons (SCS, 1980) cost \$13.9 million per year. (6) Royalty on bacteria at 6 percent of fermenter flow costs or \$2.28 million per year or assume bioaugmentation cost at \$0.008 per pound destroyed. (7) Assume methane credit to a) incinerate off-gas for volatiles or b) use in boiler as fuel at 8.5 ft^3 methane per pound COD destroyed (say10,000 mg COD/l) gives \$15.7 million credit (@ \$3.50 tcf). Table VI-2 compares these results.

Table VI-2

COMPARISON OF COST FOR LANDFILLING ETHYLENE DICHLORIDE/VINYL CHLORIDE WASTE TO ANAEROBIC FERMENTATION

Cost Basis: 120,000 metric tons/yr

Incineration	Landfill		Fermentation	
			dilution level	
			to 5,000 ppm	to 10,000 ppm
@ $300/ 1,000 lbs.*	@ $150/ 1,000 lbs.	1. Dilution water	3,170,000	1,585,000
		2. Carbon source	9,600,000	9,600,000
		3. Fermenter	38,000,000	19,000,000
		4. Sludge disposal	6,600,000	6,600,000
		5. Trickling filter	13,900,000	6,950,000
		6. Bioaugmentation or Royalty	2,280,000	2,280,000
		Subtotal	59,650,000	46,015,000
		7. Methane credit	(15,700,000)	(15,700,000)
$79,200,000/yr	$40,000,000/yr		~~57~~,850,000/yr 73	30,315,000/yr

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*Require fuel dilution due to Cl content (Wukasch, 1981)

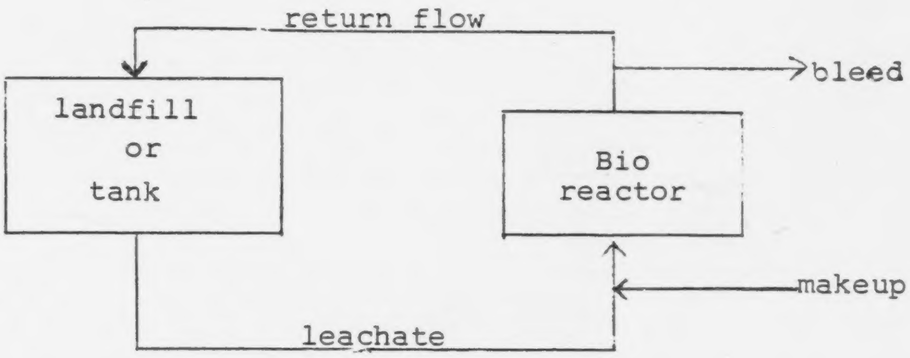
Dollar values are derived for fermenter, sludge disposal and trickling filter from SCS (1980).

(SOURCE: AmTech Consultants)

Such an alternative may not be as cost-effective nor as straightforward as indicated because concentration, system design, as well as dilution or bioaugmentation sources would be required. Furthermore, treatment efficiency and kinetics of the process would have to be established for as near complete destruction as possible including the possible use of genetic engineered organisms through bioaugmentation. Thus costs may be increased but still competitive. On the other hand, innovative techniques may be developed, decreasing costs.

Pretreatment of wastes could also be conducted to make them more amenable to biological destruction. This would include processes that either irradiate or oxidize the sludges prior to their dilution to a concentration optimum for biological treatment.

The types of biological processes that might be used were previously described; however, for insoluble organics, sludge systems such as aerobic and anaerobic digestors would be applicable, and for soluble organics fixed bed systems. Alternatively, the solid waste could be embedded and a recycle leachate stream biologically treated. Such an operation could be conducted, for example, through a contained landfill or tank.



(SOURCE: AmTech Consultants)

The advantages of tank systems, however, are found in containment and the collection of off-gas which may contain volatile toxic organics. Anaerobic systems have the advantage of producing methane from the hydrocarbons, and volatilized toxic organics would be collected simultaneously. Thus, both methane and toxic gases could be incinerated with the methane serving as the fuel source in the off gas.

Thermophilic aerobic and anaerobic systems may also be applicable for various systems as they generate a lesser amount of sludge, and for the anaerobic processes, greater amounts of methane per pound of organic carbon treated. Rates of reactions are also considerably greater for thermophilic rather than mesophilic processes, thereby decreasing retention times and capital costs (Cooney et al., 1975).

b. Wastewater Streams and Wastewater Sludges

The wastewater streams and sludges which are listed as hazardous are also potentially biodegradable, and the same form of treatment, anaerobic and aerobic, as discussed above, would be applicable. Wastewater streams bearing the toxic materials are disposed of in ponds and lagoons and are frequently injected in deep wells for ultimate disposal.* These ponds and lagoons could be upgraded by the installation of aerators and augmented by the addition of biological formulations to enhance the rate of degradation without increasing capacity. Alternatively, anaerobic or aerobic treatment in contained processes could be applied. More efficient degradation of the wastewater will decrease the toxicity of the resulting sludges.

Sludges from biological wastewater treatment systems are characterized as having an amount of the original organic chemicals converted into cellular biomass, together with toxic organics that have been adsorbed. After their production, sludges from wastewater treatment plants are further processed according to the selected mode of disposal. They can be thickened, digested, mechanically dewatered, transported, applied to land, composted, placed in landfills, dumped in the ocean, or incinerated. During these processes the sludges can change their physical, chemical, and biological properties (Bryan, 1981).

Sludges generally have a high oxygen demand and are considered appropriate for treatment through anaerobic digestion and could be accommodated onsite through the use of anaerobic digestors in their concentrated form. This term is frequently referred to as stabilization (Hartenstein, 1981). Alternatively, processes such as aerobic thermophilic digestion where pure oxygen is supplied, could be used to satisfy the oxygen demand of the concentrated material through aerobic means (Jewell et al., 1978). In either case, a stabilized sludge will result which could still have toxic materials present, even though the purpose of further degradation were to detoxify the material. Pure oxygen thermophilic aerobic digestion, however, has been reported to result in a sludge of poor quality (Etzel, 1981).

Land application and composting are frequently used methods to further decompose sludge. They are generally aerobic processes and the sludge is dispersed or tilled sufficiently to allow exposure to air through the pore space in the soil or compost. Many different types of toxic compounds can be applied to land and decomposed (Overcash, 1981) with precautions taken to assure** the assimilative capacity of the soil bacteria is greater than leaching into aquifers. Ward (1981) has studied the application of certain pesticide production wastes into soils through land application and is developing a commercial trial with a major producer. Bioaugmentation with genetically engineered microorganisms may also be used to increase the efficiency of degradation of certain specific chemicals (Chakrabarty, 1981) in soils.

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*See Table VI-1

**Land application of toxic (i.e. carcinogenic) waste is controversial

In test plots, soils contaminated with up to 1 percent of 2,4,5-T and related chemicals were shown to be detoxified quite rapidly when genetically engineered bacterial cultures were applied.

Thus, it is reasonable to assume that land application, or, analogously, composting, can be used to further biologically detoxify many types of toxic wastes, whether they be wastewater sludges or relatively concentrated organics, if applied at proper concentrations and with biological seed, as necessary. In addition, pretreatment through irradiation could also be used to render the sludge more amenable to biodegradation. When land application processes are applied it is necessary to assure that toxic waste constituents not be volatilized to the air or leached into ground waters.

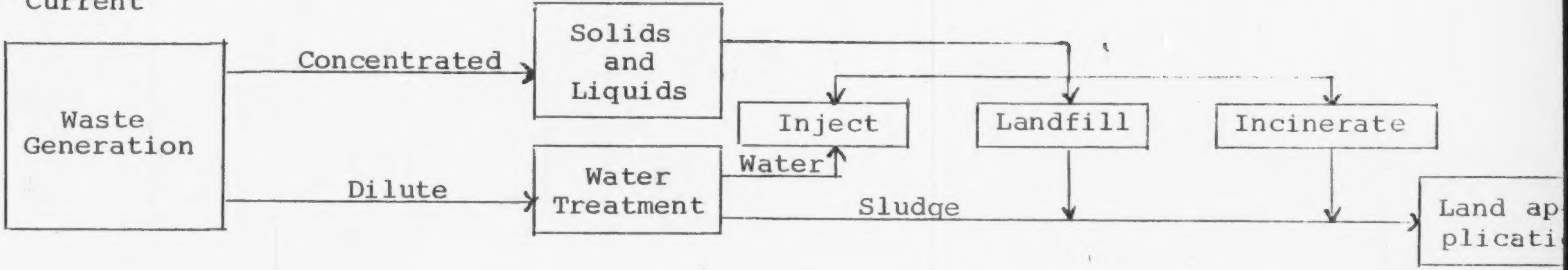
In summary, many of the factors which limit the application of biotechnologies can be overcome through pretreatments, engineering modifications to the system such as dilution or aeration, and by the use of genetically engineered bacterial formulations. As indicated in Table VI-1, nearly all of the generators of the hazardous wastes listed do not use biological processes to degrade the material and, if so, generate a sludge considered hazardous. Through upgrading or fermentations, even with highly concentrated still bottoms, biological treatment could be cost-competitive if wastewaters were available for co-disposal (or recycle systems devised) and biological formulations available for degrading particular types of toxic materials. Major opportunities are available for retrofitting existing wastewater treatment systems/lagoons and developing co-disposal techniques for concentrated solids and liquids.

Figure VI-1(a) illustrates the current state of disposal for the hazardous organic wastes in Table VI-1. Figure VI-1(b) illustrates a sequence integrating biological treatment into the process reducing landfill, injection, and incineration as alternatives. Though biological treatment holds considerable promise in reducing hazardous waste generation onsite, it is likely that many of the current practices will be required for wastes that are too costly or technically not feasible to treat biologically (e.g., due to presence of inorganics).

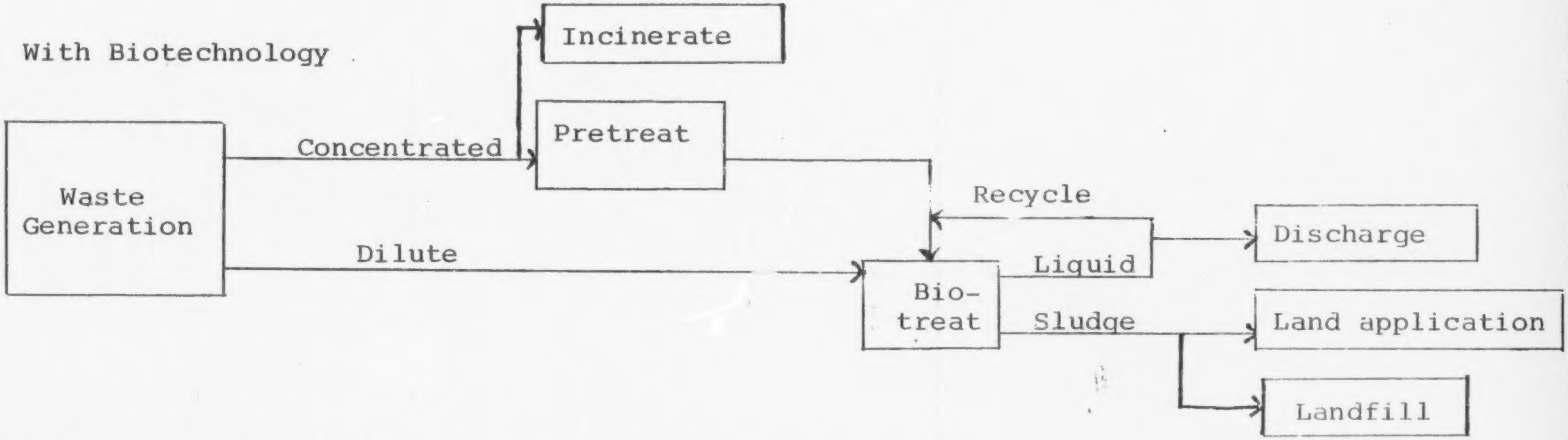
Figure VI-1

CURRENT HAZARDOUS WASTE MANAGEMENT
AND WITH BIOLOGICAL TECHNOLOGY

a. Current



b. With Biotechnology



(SOURCE: AmTech Consultants)

2. Research and Development

a. Public

Current research and development efforts in biological technologies for the control of hazardous wastes are being significantly reduced in the public sector, primarily due to the widespread budget reductions in most government sponsored R&D. These budget reductions come at a time when a major transition is taking place in the biological sciences towards the use of genetic engineering for practical applications. Because genetic engineering is a high risk/high reward research effort, such directions may not be impacted as significantly as research in conventional and traditional programs. However, in the short term, the consequences of budget reallocation may significantly impact biological technologies in hazardous waste treatment. As research needs switch from conventional pollution control to hazardous/toxic waste control, several counterproductive results can occur. For example, within the EPA Office of Research and Development, studies of the use of biological waste treatment for toxic pollution control have almost exclusively been conducted through the Municipal Environmental Research Laboratory (MERL), Biological Waste Treatment Program. This group in turn interfaces with many other EPA research efforts and program offices concerned with the control of toxic material. Their major concern is a focus on toxics in municipal landfills, to municipal sewage treatment plants, and in municipal sludge disposal land farms. Until sufficient disposal capacity is developed under RCRA Subtitle C to control the discharge of toxic materials which could take 5-10 years, these practices may continue.* Thus the group's functions are needed. However, this biological research group may be reduced in force in FY83 (Krishna, 1981). As the Industrial Environmental Research Laboratory is mostly physically/chemically directed in research, it is doubtful that priority funding would occur for biologic technology development if it were to become transferred there for a focus on industrial waste. The current program has performed some very fundamental work for the Agency over the years in biological programs.

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*Discharge into municipal sewage treatment plants might be a biological method to destroy some types of toxic waste, if diluted to a proper concentration and the toxic substrate continuously discharged through an equalization basin to the sewage system (Schroy, 1981). Legally, however, this would make the municipal sewage treatment plant a hazardous waste treatment facility and a generator of hazardous waste if present in the sludge in hazardous quantities (Shomaker, 1981).

Outside of the impacts of budgetary reductions, the promulgation of RCRA Subtitle C and related toxic materials legislations have stimulated research and development activities into new biological approaches for toxic material control. Other than the Departments of Defense and Energy, that perform research and development for their own operations, the Environmental Protection Agency, Department of Agriculture, and National Science Foundation are the major support arms for the research and development of biological technologies in toxic pollution control.

Research strategy development and the implementation of research within the Environmental Protection Agency has in the past been a fairly slow process with planning of several years before a decision is made on what research activity to initiate. Through EPA/ORD Committee structure, short term research is expected to be responsive to programmatic office needs on an annual contract basis, whereas long term, basic research is more related to internal R&D at specific Environmental Research Centers. Interaction with universities is usually supported through the competitive grants program. It is difficult to estimate the budget allocated to biological technology development even with the existing committee structures, because relevant research could be administered in a matrix fashion through the 20 EPA/ORD field offices. For example, one subcomponent of a specific office, e.g., the Biological Waste Treatment Program at MERL, Cincinnati, could be an agency-wide lead point for expertise, and funds transferred accordingly for specific research and development activities. Furthermore, programmatic offices at headquarters, require research to support regulatory criteria development, as well as regional offices to implement and enforce regulations. Thus EPA/ORD field components serve to support their own mission as well as the needs of regional and programmatic offices. The ORD/Research Committees in turn try to centrally track the budget and develop coordinated research strategies but in the past has not become too successful. The Environmental Protection Agency has often confronted criticism in this regard. An Office of Exploratory Research (OER) has been set up to maintain some lead in response to Agency forecasted needs.

Nevertheless, several Environmental Research Centers/Laboratories have become focal points for activities in biological approaches in toxicity control. These are research activities related to RCRA Subtitle C and found primarily at the Municipal Environmental Research Laboratory (MERL) and Industrial Environmental Research Laboratory (IERL) in Cincinnati, Ohio, the R.S. Kerr ERL in Ada, Oklahoma, and the Oil Hazardous Materials Spills Branch in Edison, New Jersey. IERL/MERL may also collaborate jointly or through equivalent offices in Research Triangle Park, North Carolina, or Corvallis, Oregon.

Several prime contractors and universities tend to serve these centers in areas related to biological approaches to hazardous waste control and have developed appropriate expertise (Table VI-3).

Table VI-3

MAJOR GOVERNMENT PRIVATE CONTRACTORS AND UNIVERSITIES IN RCRA-SUBTITLE C RELATED BIOLOGICAL TECHNOLOGY R&D

Group	General Area	Support
Private Contractors		
Midwest Research Institute	Biodegradation	MERL/IERL
Tetra Tech	Bioassay	Programmatic Offices
JRB Associates	Uncontrolled sites	Headquarters
SRI International	General	Headquarters
AD Little	General	Headquarters
Weston	Uncontrolled sites	Programmatic Offices
Black & Veach	Uncontrolled sites	Programmatic Offices
Camp, Dresser, McKey	Uncontrolled sites	Programmatic Offices
Mitre	General	Headquarters
Versar	General	Headquarters
Universities		
Louisiana State	General	EPA
MIT	General	NSF/EPA
North Carolina State	Land farming	EPA/MERL
Notre Dame	General	NSF/EPA
Purdue	General	NSF/EPA
Rutgers	General	MERL
Stanford	Groundwater	NSF/EPA
University of Illinois	Genetic Engineering/General	EPA
University of Illinois (Medical)	Genetic Engineering	NSF/EPA
University of Oklahoma	Bioreactors	EPA
Texas A&M	Land Systems	EPA

(SOURCE: AmTech Consultants)

The Environmental Protection Agency's Office of Exploratory Research has established a planning program at the Advanced Environmental Control Technology Research Center (University of Illinois) to evaluate applications of genetic engineering in the pollution area.

This program has served three prime functions in EPA's current planning (Johnson, 1981).

- Identification of limits to biodegradation
- Gene manipulation methods
- Identification of genetic engineering for pollution control

Table VI-4 describes the results of a survey by AECTRC with Environmental Protection Agency personnel on situations that either have no economically feasible solutions or that might plausibly be addressed by the genetic engineering of microbes (Johnson, 1981). These applications not only require the genetic manipulation and development of a microbe, but also the appropriate technology engineering to maintain the organisms' viability in the affected environment. Their recommendations are being used to develop EPA/ORD planning (Levine,1981).

The efficiency of the use of commercial biological formulations in the treatment of toxic materials has not been reported in a peer reviewed journal (Johnson, 1981); however, data supplied by manufacturers for treatment of conventional pollutants in industries known to discharge toxic wastes have shown considerable improvement in performance (McDowell, 1980; Thibault, 1979). Nevertheless, both the industry (Polybac, 1981) as well as researchers (Johnson, 1981) feel that a controlled study should be conducted on the applications of available commercial formulations. The industry feels the technology is here today where researchers often state five years at a minimum (BioTechnica International, 1981).

EPA (Levine, 1981) thinks that basic research is the appropriate direction ORD should take in biotechnology (sic. genetic engineering) focusing on applied genetics. This would be an emphasis on the development of cell lines by contractors. The question remains, however, what an agency would do if it were to develop a cell line. Under recent revisions to the Patent Laws, small businesses and universities are to retain title to inventions developed under government funds.

Table VI-4

ENVIRONMENTAL PROBLEMS POTENIALLY SOLVABLE BY APPLIED GENETICS (Johnson, 1981)

1. Contained Waste Streams
    - a. Generic improvements in biological treatment including decreased treatment time, improved effluent BOD, reduced sludge volumes
    - b. Specific improvements
        - i. NH_3 removal
        - ii. Phosphate sequestration (luxury uptake of P)
        - iii. Improved flocculation
        - iv. Degradation or detoxification of particular components of a waste stream, e.g., cyanide, haloaromatics, organometallics
2. *In situ* Treatment of Pollution
    - a. Treat contaminated soils (N.C. roadsides - PCBs)
    - b. Treat contaminated sediments (kepone in James River)
    - c. Treat marine oil spills
    - d. Regeneration of physical adsorbents (kepone-laden activated carbon)
    - e. Hazardous waste landfills
3. Miscellaneous
    - a. Specific diagnostic methods to assay waste components
    - b. Highly speculative: e.g., air pollution abatement

The trend in applied genetic engineering (Vi Nosa, 1981) is toward the development of a microbe containing one plasmid with the total genetic sequence incorporated/cloned to effect complete mineralization of a specific toxic pollutant. This would involve possibly 10-20 genetically coded enzyme systems cloned onto one plasmid. The rationale here is that because plasmids interchange between microbial species, the plasmids' introduction into a hazardous waste contaminated location can naturally transfer the same genetic properties through natural processes into other native microorganisms. Thus, the theory holds that if one were to obtain a plasmid through genetic engineering capable of degrading each major priority/toxic pollutant, a mixed bacterial culture innoculated into a site should effect the decomposition of whatever toxic materials that are cloned without allowing for natural evolutionary processes to accomplish this feat.

Research is being conducted at several institutions around the country on plasmid techniques in pollution control. Pierce et al., (1981) has been identifying and isolating plasmids from environmental strains of bacteria capable of efficiently degrading 2,4-D. Recently, Chatterjee (in press), under funding from several government agencies including NSF and EPA, reported the development of a pure culture of bacteria (AC1100) capable of the complete mineralization of the herbicide 2,4,5-T (Agent Orange). Previously, no pure cultures were known to be capable of utilizing 2,4,5-T as a sole source of carbon and energy (Rosenberg and Alexander, 1980). AC1100 is capable of utilizing 2,4,5-T at extremely high concentrations (0.1-2 percent). The isolation took several years and involved the selection and manipulation of plasmid based genetic material from several strains capable of utilizing individual components in the degradative sequence (i.e., Pseudomonas, Arthrobacter, Klubicella, and Acineobacter). The technique has been termed Plasmid Assisted Molecular Breeding (PAMB) (Kellogg, 1981).

Through the above work several plasmids have been isolated and identified for their role in the total bioconversion of chlorinated benzoates and biphenyls.

Plasmid	Reaction
TOL	4Cba ---> 4-chlorocatechol
	3,5Dcb ---> 3,5-dichlorocatechol
pAC25	4-chlorocatechol ---> CO_2+H_2O+HCl
pAC28, pAC29	3,5-dichlorocatechol ---> CO_2+H_2O+HCl
pAC28, pAC29, TOL	3,5-Dcb---> CO_2+H_2O+HCl
pM5	3,5 DCB---> 3,5-Dcb

Nomenclature

Dcb Dichlorobenzoate   Cba chlorobenzoic acid   DCB Dichlorobiphenyl

The overall significance of these genetic engineering developments and their potential applications are not clear. Recent field tests indicated excellent performance of AC1100 in a garden plot (Chakrabarty, 1981a). However, no applications have been made under controlled bioreactor conditions. The use of these cultures may be only applicable to cases where the substrate exists as a relatively neat diluted solution. With 2,4,5-T a problem is toxic contaminants in existing government stockpiles, and an organism would have to be developed to degrade these along with 2,4,5-T (ViNosa, 1981). Other activities within EPA involve biological reactor design. For engineering technology to be developed, a cell culture must be available and kinetics established prior to the design of engineering hardware at EPA/MERL's Cincinnati pilot plant facilities (Smith, 1981).

Other areas of research include the development of ionizing radiation systems to sterilize pathogen-containing sludge (DOE, 1980) and wastewater (MIT, 1979). These processes may have application in treating leachate or sludge that contain genetically engineered organisms to affect their viability before release into an open environment. Because, as previously mentioned, ionizing radiation can also break down the complexity of recalcitrant organic molecules to render them more biodegradable, it would seem plausible to develop a system, such as for a land farm, that ionizes to set up a material for biodegradation followed by seeding. Done on a periodic (annual) basis, more rapid degradation and increased loadings might be effected.

Other areas, such as thermophilic anaerobic digestion and immobilized cell systems, are appearing as applied research efforts in the biological technology area (Peyton, 1981; Scott, 1981; Lee, 1981).

In general, however, the Environmental Protection Agency's current programmatic research efforts are more towards the development of physical/chemical methods where there is a high degree of reliability, predictability and performance efficiency. As previously mentioned, these may not be cost-effective in comparison to some biological technologies. Table VI-5 summarizes government research activities in biotechnology applications to hazardous waste.

b. Private Research

Private research in biological technology for pollution control is conducted in university laboratories, by private contractors, entrepreneurs, companies that discharge waste, and venture capital firms. Though basic research is sponsored with long term goals, such as in the genetic engineering venture companies or universities, most research is short term, applied, and responsive to an industry's need and regulatory compliance requirement. Research in industry is proprietary, with maximum return on investments the goal, and many techniques that are developed can be protected as trade secrets or license. Thus, in the formative stages little information is known.

Table VI- 5

MAJOR GOVERNMENT RESEARCH AND DEVELOPMENT FOR BIOLOGICAL TECHNOLOGIES IN CONTROL OF HAZARDOUS WASTE

Agency	General Area	R&D Activity
1. Environmental Protection Agency		
a. Edison, New Jersey/MERL	Spills	• Feasibility of in-situ biological treatment of aquifers (FY 83)
		• Biological treatment of surface runoff (FY 82)
		• Novel biodegradation processes/ pure culture identification (FY 81)
		• Microbiological countermeasures for spills on water (continuing)
b. Cincinnati, OH/MERL	Biological Waste Treatment	• Secondary biological treatment feasibility
		• Biodgradation screening studies of toxic pollutants by activated sewage sludge (FY 81)
		• Landfarming of sewage sludge with toxic organics (FY 82)
		• Phosphorus removal by use of genetic engineering (FY 83)
	Landfills Uncontrolled	• Biological leachate treatment systems (FY 82)
		• Studies related to impacts of co-disposal (FY 82)

(SOURCE: AmTech Consultants)

Table VI-5 (Continued)

Agency	General Area	R&D Activity
1. EPA (cont.)		
c. Cincinnati, OH/MERL-IERL	Biotechnology development	● Pilot plant treatment center
d. Cincinnati, OH/IERL	Genetic Eng.	● Dioxin/2,4,5-T degradation ● Misc. toxics degradation ● General strategy development (FY 82, Table VI-4)
e. Ada, Oklahoma, R.S. Kerr Laboratory	Land/groundwater systems	● Microbial fate processes in soil/aquifer systems (FY 82) ● Landfarming (FY82)
f. Washington/ORD	Genetic Eng.	● Health and environmental effects (competitive grants, FY 81)
g. RTP/IERL	Industrial Wastewater	● Bioassay systems (FY 81) ● Genetic eng. control technology
h. ERC's	Biological processes	● Misc. studies on control technolog fate and effects processes
2. Department of Agriculture		
a. Beltsville, MD	Composting	● Fate and degradation of misc. toxi compounds (FY 82)
	Photolysis	● Biodegradation in combination with irradiation (FY 82)

(SOURCE: AmTech Consultants)

Table VI-5 (Continued)

Agency	General Area	R&D Activity
3. National Science Foundation	Genetic Eng.	● Halogenated pollutant biodegradation (FY 82)
		● Aliphatic biodegradation (FY 82)
		● Microbial mercury resistance (FY 82)
	Environmental Engineering	● Sludge management (FY 81)
		● Innovative technologies (FY 81)
		● Disinfection (FY 81)
4. State Programs and Activities	RCRA-Subtitle C	● Misc., through EPA state grant programs (Skinner, 1981)
5. Department of Energy		
a. Oak Ridge National Lab	Technology Development	● Immobilized cell systems, fixed and fluidized beds
		● Aerobic and anaerobic applications
b. Sandia Laboratories	Sludge	● Cs-137 sludge irradiation

(SOURCE: AmTech Consultants)

In general, however, the private research funding in biotechnologies for toxics control is more a direct function of the level of regulatory compliance rather than in normal market forces, regulations or awareness of liability and health risks creating the incentive for R&D in pollution control.

On a time scale, once a market incentive is developed in industry, for example in the case of a spill, unique research can be conducted in a matter of a few weeks with an appropriate solution for field application the outcome (OH Materials, 1981). Though sometimes termed laboratory/bench-scale feasibility, these activities are essentially applied R&D in response to an imminent health threat.

Research and development is being conducted by major trade associations on topics that are of general concern to the industry. This research is generally in response to proposed rules and regulations.

The American Petroleum Institute (Rucker, 1981) is currently conducting four studies all related to land treatment of oil refinery waste. The studies take on added significance in respect to the 101 of 150 land farm sites in the country that treat primarily refinery sludge.

- Emission of volatile hydrocarbons from land farms
- Land farm site and fate analysis of organic materials
- Closure procedures for land farms
- Unsaturated zone lysimeter testing of land farms

These studies will help to determine proper loading rates, impacts from atmospheric emissions, potential contamination processes to underlying aquifers, and health implications of closure procedures.

Many chemical industries treat their wastewater biologically onsite and the resulting sludge is considered hazardous if spilled commercial products or residues are present, or if it is specifically listed as a hazardous waste. The organic chemicals industry (organic, synthetics and plastics industry), as represented by the CMA and five CMA member plants, has agreed to furnish EPA with samples of untreated and biologically treated effluents from biological treatment processes. Over 50 chemicals are being evaluated (Romano, 1981). Preliminary data show that greater than 90 percent removal from the water stream is occurring, but whether this is due to stripping, degradation, or accumulation on the sludge is not known. It is likely, however, that the majority of the material is being biologically degraded. Future research efforts will depend upon the results of this study (Romano, 1981).

The potential for decreasing the generation of hazardous waste through better biological treatment efficiency is demonstrated by research recently conducted by Polybac Corporation (Krupka, 1981). In this instance a solvent previously thought recalcitrant by the synthetic rubber industry was shown to be 95 percent decomposed in a half-day pilot reactor system when treated with a bacterial formulation, Hydrobac. The research indicated that the material, morpholone, previously thought to be recalcitrant and requiring disposal as a hazardous waste, could now be treated biologically onsite. Existing applications by the chemical industry indicate that, when properly designed, a biological reactor should be able to treat most organic wastes, thus eliminating any hazardous sludge for RCRA Subtitle C disposal. For example, Monsanto Chemical (Schroy, 1981) had designed a system for decomposing biologically paranitrophenol in the early 1960's quite successfully. The aerobic system, however, was based on an eight-day retention, a design basis that would not be used today for cost reasons (half to one day are more normal).

Many members of the chemical industry feel that because of the biomass present in sewage treatment plants, sewage treatment plants should be able to biodegrade toxic chemicals when they are diluted to non-toxic concentrations and continuously supplied.

There are advocates and adversaries within the industrial community on the practicality and efficacy of using biological formulations for toxic biodegradation. Again the argument is strong that, if properly designed and operated, a recalcitrant waste should be degraded, and bacteria that acclimate/evolve in the lab (for biological augmentation purposes) should also be able to acclimate/evolve in the field (the Ubiquity Principle). DuPont and Dow as well as Monsanto have all had considerable success in biological treatment of toxic wastes (Cambell, 1981; Daniels, 1982; Schroy, 1981).

All of these companies continue their own research on waste treatment systems, and in some instances commercially market their expertise or processes. For example, environmental engineers at Monsanto design biological systems for the petrochemical industry and DuPont sells systems such as their Powdered Activated Carbon (PAC) process that serves as a fluid media for microbes. Celanese markets an anaerobic filter process for petrochemical wastes.

Economics may preclude designing a system based upon the most recalcitrant organic waste due to capital or space limitations, or adding on to a system for new types of organic contaminants, and in these instances advocates of biological augmentation can be found. For example, at Exxon's Baton Rouge Petrochemical Plant, the opportunity for a parallel train study on the UNOX oxygen activated sludge system occurred. In this instance a system receiving over 4 MGD with a daily maintenance of 4 lbs. of a formulation, Phenobac*, as compared to a control, demonstrated an increase in kinetics of over 2.5 and reduction of Total Oxygen Demand (TOD) of 46 percent, at the median level (Thibault, 1979).

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*TM

In the field of bioaugmentation (addition of acclimated biomass to bioreactors) a major emphasis is now being placed on the development of commercial formulations that degrade toxic organic material for use in spills and chemical bioreactors. Information received by Polybac Corporation (McDowell, 1981) shows that their commercial formulation can grow on 17 toxic organics as a sole source of carbon source (as presented in II.A.4).

Other information published in newsletters and from Polybac to the Congressional OTA (Zitrides, 1981) have indicated their formulation can degrade over 80 compounds listed by EPA from partial to complete mineralization. This is a subject of continuing research on the part of Polybac.

Sybron Chemicals, another major supplier of bacterial formulations, is conducting research on products that degrade halogenated aromatic and aliphatic compounds (Telepchak, 1981) and expect to have formulations available for application to spills within a year.

Microlife, Inc., is also conducting rDNA research on toxic biodegrading organisms (Glendening, 1981). Many other small developers in biological formulations are coming into the business according to the current major suppliers, mostly around university communities (Krupka, 1981).

Several of the major biotechnology venture companies are also performing research in pollution control applications, presumably in genetic engineering applications (First, 1981). The firms are quite hesitant to indicate in what directions they are applying biotechnology.

Company	Location
Biogen, S.A.	Switzerland
Biotech, Inc.	Madison, WI
DNA Science, Inc.	New York City
Ecoenergetics, Inc.	Fairfield, CA
Microlife Technics, Inc.	Sarasota, FL

(SOURCE: First, 1981)

A considerable amount of research is being conducted by companies involved more in the development and application of the hardware and machinery that are the basis for containing and using the microbial systems previously referred to. They can be biotechnology companies focusing on fermentation or the same companies that provide the field services presented in the market profile section and concentrate more specifically on the technology. Most every large architectural engineering firm, i.e., Bechtel, Fluor, or those specializing in applications for waste management, i.e., Camp, Dresser, Mckee, Law Engineering, Aware, Inc., Metcalf and Eddy, etc., have process technology departments in research and engineering and routinely develop or survey new techniques dependent upon their client's particular need.

Most major industries in the country today have either a direct or indirect interest in biotechnology research and development. These include most major oil, petrochemical, chemical, pharmaceutical, and food companies and it is likely that some of their efforts are focused on internal effluent/hazardous waste biological treatment techniques. For the most part, however, their investments in biological technologies have been with risk capital directed towards agricultural, biomedical, and chemical feedstock (e.g., biomass conversion) and discussions with several of these companies indicate that their pollution research and applied genetics programs are totally separate efforts (industrial contacts, 1981). It appears that if specific applications, needs, or incentives were provided they would be very well equipped to respond; however, the pollution control sector is not often considered a "glamour industry" to the classical genetic engineering community, looking for high reward pay-offs in the fields mentioned. Pollution control, specifically hazardous waste, is a problem of national importance and solutions other than landfilling are required. Some companies that have reported activities in applied genetics for hazardous waste treatment are Du Pont, Hooker Chemical (with Notre Dame), Koppers Company (with Genex), and Mead Paper (with Stanford) (OTA, 1981).

B. LEGISLATIVE AND INSTITUTIONAL

1. Legislations and Regulations*

Biological technologies for the control of hazardous waste under the Resource Conservation and Recovery Act (RCRA) are closely related to many other legislations concerning toxic wastes, and can be influenced accordingly. The Resource Conservative and Recovery Act (PL94-580) was enacted on October 21, 1976, to provide, among other matters associated with the recovery of resources from discarded materials, enabling legislation for the Environmental Protection Agency to control the management of hazardous waste. This section of the law is referred to as Subtitle C--Hazardous Waste Management. As evidenced by the Agency's simultaneous issuance of the Consolidated Permit Program with RCRA Subtitle C regulation on May 19, 1980 (45FR33287), the management of hazardous waste under RCRA is closely related to other industrial waste treatments of toxic materials (e.g., Clean Water Act PL95-217 - Sec. 307, NPDES). For example, industrial waste water treatment facilities that generate a sludge containing toxic chemicals can be considered, under RCRA, a generator of hazardous waste, and require permitting for off-site disposal of this sludge. Additionally, the Consolidated Permit Program also considered simultaneous permitting for the Underground Injection Control (UIC) of toxic materials into potable aquifers (SDWA, PL95-190), and Prevention of Significant Deterioration (PSD) under the Clean Air Act (CAA, PL88-206). In essence, RCRA Subtitle C became a regulatory cornerstone to contain, recycle or treat all toxic material discharge from an industrial facility.

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*In this chapter "FR" refers to the Federal Register. A designation (e.g., 45FR33287) refers to Volume 45, Federal Register, Page 33287. "CFR" refers to Code of Federal Regulations. A designation (e.g., 40CFR261) refers to Title 40, Code of Federal Regulations, Part 261. All "FR" and "CFR" citations in this section can be found in the Federal Register, Volume 45, Books 1, 2 and 3, May 19, 1980.

Additionally, the development of regulations for management of toxic waste under RCRA Subtitle C revealed a considerable amount of toxic material of the type the regulation was to control, heretofore stored or disposed of in uncontrolled landfills, storage yards, or dumped with innocuous materials. With the realization of the cost of cleanup and potential threat to public health these situations posed, additional legislation was promulgated by Congress, the Comprehensive Environmental Response, Compensation, and Liaibility Act of 1980 (CERCLA, PL96-510), levying a tax and liability provision on industry for the cost of remedial action at such sites. Finally, the Agency also realized that much of the above disposal was technically and procedurally similar to the cleanup of toxic material spills as legislated for control under Section 311 of the Clean Water Act.

Nearly all toxic materials generated in our economy that are not destroyed in normal use will require ultimate disposal in a RCRA permitted treatment and disposal facility. Thus the potential sources of toxic waste are ubiquitous under RCRA definition. For example, though the Toxic Substances Control Act's (TSCA, PL94-469) emphasis is upon screening and licensing new chemicals in commerce under premanufacturing notification rules, it can control existing toxic chemicals associated with the above legislations through its imminent hazards provisions (Section 7) (Bond, 1981). Because the product eventually requires disposal after its use, and the manufacturing process produces a waste, TSCA has to consider these elements in its screening process. TSCA's imminent hazard provision is only enforced as a catch-all, for example, with polychlorinated biphenyls (PCBs), disposal of defense stockpiles of nerve gas or dioxin contaminated herbicides. PCB disposal is planned to be transferred from TSCA to RCRA regulated authority. FIFRA (PL92-516), the Federal Insecticide, Fungicide, and Rodenticide Act, is similar to TSCA as its emphasis is on licensing pesticides for commercial use but also contains provisions for safe disposal (Section 19).

a. Clean Water Act

Effluent Guidelines Regulations under the Clean Water Act have an influence on the development of on-site biological technologies for the treatment of RCRA type organic wastes. These regulations are essentially a recap of the best available technologies (BAT) for a particular category of industries and are a direct reflection of industrial status in the use of various types of treatment technologies. Biological methods have widespread use in reducing dilute concentrations of organic materials, and particularly toxic organic chemical compounds in the organic chemical and pesticides industry. Nevertheless, compliance to BAT is only required under an NPDES permit, and otherwise industry is allowed to discharge its waste to a publicly-owned treatment works (POTW - sewage treatment plant) where, in turn, the local municipality must obtain an NPDES permit. It is between the municipality and industrial discharger on a state or local level to develop pretreatment requirements. Naturally, the concern here is the ultimate biodegradation of toxics in the POTW and the disposition of any toxic organics remaining in the sludge and its use in land farming. RCRA does not control the discharge of toxics to POTWs but could possibly regulate the use of the sludge.

In spill control and cleanup, regulations are generally directed towards removing and decontaminating a spill site as rapidly as possible. Generally, any technology that can perform the desired objective is considered for use. Annex X of the National Oil and Hazardous Substances Pollution Contingency Plan considers the use of additives to assist in the clean-up of spills. No provisions are specifically available for biological methods, though the techniques are used in the private sector. Annex X will be updated to include biological materials (Dorrler, 1981). However, acceptance has already been provided to several biological formulations for use in spills (Biglane, 1980) at the product data stage.

Finally, municipal sewage treatment plants are a receptacle of hazardous wastes and are not covered in the regulations. There is no provision controlling the discharge in either RCRA or through the consolidated permit process for prohibiting these discharges (45 FR 33097, 33171) unless the state or municipality requires pretreatment standards. One provision in the permit process that could have a major influence on the use of biological technologies is the requirement in Section 304 of the Clean Water Act for Best Management Practices (BMP). Under Section 304, existing biological treatment plants (RCRA facilities as well as NPDES permitted bioreactors and POTWs receiving toxics) could be influenced to use biological manipulation methods for degrading toxic waste materials more efficiently.

b. CERCLA

As designated in Section 106 of CERCLA, abandoned hazardous waste sites are regulated, not in conformance to RCRA Subtitle C standards as treatment, storage, disposal facilities, but as designated in the National Contingency Plans under the spill provisions of Section 311(c)(2), 308, 309 and 504(a) of the Clean Water Act; and other imminent hazard and related provisions controlling toxic contamination in the major acts.

However, as designated in the contaminated spill materials provision of RCRA, these CERCLA sites could be classified as generators of hazardous waste and thus any removed material must be sent to RCRA Subtitle C permitted treatment storage, and disposal facilities. If this treatment were to occur onsite, compliance with the RCRA facility standards and permitting provisions should, in principle, also be required. Thus, the application of biological technologies is also relevant under this regulatory interpretation of CERCLA to include controlled cleanup of previously uncontrolled hazardous waste sites.

c. RCRA (FR, 1980)

Specifically, RCRA Subtitle C regulations are divided into five key parts, namely,

1. Identification and listing of hazardous wastes (40 CFR 261)
2. Standards applicable to generators of hazardous wastes (40 CFR 262)
3. Standards applicable to transporters of hazardous wastes (40 CFR 263)
4. Interim status standards and standards applicable to owners and operators of hazardous waste treatment, storage and disposal facilities (40 CFR 264, 265)
5. Permits for treatment, storage, and disposal of hazardous wastes (40 CFR 122 and 124)

Though listing of a hazardous waste can be voluntary on the part of a generator (40 CFR 262.11(c)), only the solid waste listed as hazardous by the Administrator (40 CFR 261 Subpart D) or under an authorized state hazardous waste program (40 CFR 123) is subject to enforcement. The listed generator has the right to petition this listing (40 CFR 260.20 and 260.22). Non-specific and specific sources of waste can be added or deleted by the EPA Administrator or state. Toxicity is the prevailing determinant for listing a solid waste as hazardous. Commercial chemical products can also be classified as hazardous wastes, though not listed as above. These apply when a chemical is generated/stored onsite at quantities greater than one metric ton per month (for 239 chemicals). There are exclusions to this quantity and apply to an additional 122 specific acutely hazardous commercial chemical products (at 1 kg), off specification species (at 1 kg), containers (at 10 kg), and spill residues thereof (at 100 kg) from any source.

Most of these compounds listed as toxic are organic and potentially biodegradable.

A generator of hazardous waste (40 CFR 262) who treats, stores or disposes of all of his waste onsite must comply with standards and interim status standards for treatment, storage, and disposal facilities (40 CFR 264, 265) and obtain a permit to do so (40 CFR 122), unless his on-site treatment is totally enclosed and part of the manufacturing process. Likewise, an off-site treatment, storage, and disposal facility receiving hazardous waste from a generator must also apply for a permit and comply with the stated standards. Thus, on- and off-site facilities and technologies are no different in principle, but in practice off-site facilities are likely to be qualitatively different as they accept waste that is only cost-effectively transportable and in compliance with equivalent Department of Transportation rules presented in 40 CFR 263 for the transport of hazardous waste. Procedurally complex permitting of new facilities may discourage some generators to install on-site technologies.

Standards for the operation of biological technologies for containing hazardous wastes are provided in the interim status (40 CFR 265) and general status (40 CFR 264) standards. Technical operating standards are established as general status standards and have not been established under Phase I of RCRA. Their development under Phase II could profoundly influence the use of biological technologies, but it is likely that these standards will be developed only on a case by case basis as permitting allows (40 CFR 122).

Under the interim status standards applicable in Phase I, all existing facilities receive interim status by filing Part A until the permit receives examination. Part B examinations are under way (Shomaker, 1981).

The types of biological treatment and disposal technologies presented in the regulations are provided with the following codes:

T67	Activated sludge
T68	Aerobic lagoon
T69	Aerobic tank
T70	Anaerobic lagoon
T71	Composting
T72	Septic tank
T73	Spray irrigation
T74	Thickening filter
T75	Trickling filter
T76	Waste stabilization pond
T77	Other
D81	Land fill
D82	Land treatment
D84	Surface impoundment

The general operating requirements for land fills and for chemical, physical, and biological processes, have a negative influence on the use of some biological technologies. For land fills, biologically immobilized, attenuated, waste appears specifically excluded as an allowable form. Impermeable liners are required but over time these may fail and release toxic materials. As leachate collection is required in accordance with the facilities' post-closure NPDES permit, a form of biological barrier might be appropriate to promote biodegradation in situ (Kirsch, 1981). This was also suggested by EPA/ORD (Table VI-4). Furthermore, the land fill regulations appear directed towards a "sterile" environment. Studies (Wilson, 1981; Shomaker, 1981) have shown that biodegradation of hazardous waste in land fills may occur under controlled conditions. Given the time and space allowance for land fills, it would seem reasonable to biologically "seed" the land fill system with genetically engineered organisms so that degradation of some toxic waste forms may be initiated rather than just allowing the toxicity to be contained until the system fails in the future. There are stringent post-closure liability requirements under RCRA for land fills, and biological seeding would be practical to reduce this liability rather than to perpetuate it into the future.

The general operating requirements for biological facilities appear to bias the use of enclosed systems that might anaerobically generate methane gas as a final degradation product.

Chemical, Physical, Biological (265.401 & 265.17(b))	Limits generation of extreme pressure, uncontrolled toxic fumes and flammable gases

The standard is not directed toward the use of anaerobic systems but for any system where incompatible or reactive waste generate extreme pressure, uncontrolled toxic vapor or flammable fumes. Nevetheless, it is important for the Environmental Protection Agency to clarify the application of this requirement to anaerobic treatment systems.

clarif

2. Insitutional Barriers

Many barriers confront the development and application of biological technologies that are heightened in the pollution control sector due to somewhat of a reliance of market forces on legislation before a user will account for the technologies market value. Most all barriers result in an institutionalized form which can be defined as a common collective concern to preserve or create policy and practices to the maximum benefit of those with common interests. Insitutional barriers can be organized or impromptu and transect all of society encompassing factors associated with social, religious, economic, industrial, educational and political concerns.

Table VI-6 presents some of these concerns around which institutional barriers can develop to affect the application of biological technologies in hazardous waste control.

Table VI-6

INSTITUTIONAL FACTORS THAT AFFECT THE DEVELOPMENT OF BIOLOGICAL TECHNOLOGIES IN HAZARDOUS WASTE CONTROL

Economic

- Contribution to the national economy
- Return on investments
- Capital and financing requirements
- Risk in performance

Educational

- Professional attitudes
- Professional skills
- User interest

Industrial

- Cost effectiveness
- Competing technologies
- Perceived need
- Research and development
- Resource requirements

Political

- Bureaucracy
- Legislations
- Lobbies
- Regulatory requirements
- Regulatory enforcement
- Research and development appropriations

Social

- Ethical concerns with genetic engineering
- Public health concerns with the use of living organisms

(SOURCE: AmTech Consultants)

a. Economic

An overriding concern and the most difficult to answer is the contribution of the technology to the national economy in terms of jobs, productivity and indirect benefits to other sectors. Because the hazardous waste market is expected to expand over the next decade, developments in this sector should not displace jobs but should create new employment opportunities. One barrier, however, is the priority pollution abatement has in contrast, for example, to developing more efficient automobiles or manufacturing processes that increases the productivity of the work force and in turn decrease the cost of consumer goods. Though pollution abatement of hazardous wastes represents a national concern, the result, being clean water or solids, cannot be represented in the same manner as a tangible commodity. Nonetheless, clean natural resources are products of the application of the technology and provides for the reduction of liability to the user.

Biological technologies are at an early stage of development for hazardous wastes and though the potential is large for its application, sufficient information has not been acquired to estimate the return on investments and capital requirements for the technology in comparison to current disposal techniques such as injection, landfills, or incineration. In general, however, the costs of biological technologies are expected to be less. As new techniques and cell lines become available for application to specific hazardous wastes, accurate cost estimates should develop. However, because of the general absence of biotechnologies in treating recalcitrant hazardous waste, most consulting engineers do not consider them in their current cost estimates of alternative approaches to potential industrial users.

Many biotechnologists feel that a primary barrier exists on the part of design engineers who emphasize systems that are more hardware and mechanically oriented, rather than on approaches that are biological and are not as complex in design (Polybac, 1981). This argument has some merit on an economic and educational basis. Design engineers' services are frequently rewarded in direct relation to the complexity and amount of equipment. Biological systems that rely upon the microbial aspects of the process do not require as much bulk capital hardware but an emphasis on monitoring and correcting its performance in operation, though automating this feature could be capital intensive (Celanese, 1981). Also, design engineers may have not been trained in or do not understand the operational requirements for microbial processes, therefore relying on physical/chemical approaches, where design parameters have been well defined.

Naturally, in the absence of design parameters to assure some reliability in performance, biological approaches have frequently been considered as risky alternatives. There are many instances where biological systems have been designed but have failed to perform properly (Union Carbide, 1972). Though it is true that biological systems are more sensitive to changes in quality and quantity of waste than non-biological approaches, many failures have been over publicized and are frequently cited as "showstoppers" by competitors in preference to alternative non-biological approaches.

On the other hand, the use of biological formulations as a waste treatment "panacea" received considerabl bad publicity in the 1960's due to poor quality material on the market (Zitrides, 1981). With increasing knowledge of the operational aspects of biological technologies by the engineering community and demonstration of their cost-effectiveness in the pollution and the allied commercial product sector, greater reliability in system design and operation should be assured in the future. It has been stated that the Japanese are further ahead of America in engineering biotechnology (fermentation technology), and though we excel in the genetic engineering of cell lines or biology of the system (Chevron, 1981), some of their engineering approaches may be applicable. Our pharmaceutical industry is, however, highly advanced and, if given the incentive, could apply their hardware and software knowledge to this sector (Etzel, 1981).

b. Educational

Generally in the United States there is a professional disparity between engineers who design a system and biologists who know about the basis for the process biology. Most universities are developing or have ongoing programs that integrate the two disciplines as "biotechnology, bioengineering, or biochemical engineering." In industry today most professionals were educated when the engineer was in the factory or on the drawing board and the biologist in the laboratory or researching literature. Thus, while the applied biologist desires to commercialize his newly found biotechnology (e.g., cell lines) to obtain the rewards of his labor, the engineer wants to be educated by him to sell his next process using the microorganisms. Consequently, professional barriers appear strong and communication on corresponding needs between professions minimal which could limit large-scale applications of biological technologies in preference for small fermentations performed in the laboratory that produce highly valuable products (e.g., interferon). It is obvious that a multidisciplinary team approach is required for large-scale biotechnology applications in hazardous waste control; however, communication appears minimal though the potential is large. For example, a recent survey of over 100 venture capital genetic engineering firms indicated that less than 10 were involved in pollution control (First, 1981).

Personal inquiry with these indicated that only several were conducting research on specific genetic engineering bioaugmentation applications to hazardous organic waste.

The availability of professional skills for educational training may also be a major barrier. Many educational institutions have lost biological expertise to major contract research institutions and consequently the impact on the supply of qualified professional labor may be significant. However, contract seminars and short-term training courses should be able to supply some of the skills that are necessary to promote particular biotechnology applications.

Due to some of the professional attitudes cited above, there may also be a general disinterest in the potential use of biological applications on the part of users in favor of more familiar physical/chemical approaches.

c. Industrial

Establishing a need for a technology is the first step an industry makes when beginning its decision process. Though pollution abatement does not directly enhance the value of the goods the manufacturer produces, public image and concern for their health and safety are motivating factors that can assist sales unless regulatory requirements or potential liabilities necessitate an action. Next in the decision making process is the selection of a technology which is most often based upon its cost-effectiveness in line with competing technologies. Factors that are usually taken into consideration at this stage are resource requirements, demonstrated performance, the reputation of the sales firm, quality of the service being purchased, and whether or not an equivalent solution can be developed using internal capabilities or process modification. Propriety is critical to all new technology developments.

Major factors that currently make up the barriers from the industrial perspective on the use of biological technology is the inability to convincingly demonstrate the cost-effectiveness of any proposed application. In contrast to competing technologies, the biological approaches cannot be taken off the shelf for application today and require some degree of research, development and pilot plant operation onsite. Confronted with this, some industries may, in turn, be more inclined to provide the waste to a hauler where the cost can be well defined, and the burden of disposal placed off the industry's shoulder. This alternative may not be cost-effective in the long run, thus investments on the part of private industry to develop biological technologies, particularly large generators, either in joint ventures with consulting engineers, entrepreneurs, or internally, are critical to the development of a biological approach.

d. Political

Political factors and the outcome of associated negotiations and policies have major influence on the development of biological technology. As the application is directed towards hazardous waste control, it is necessary that legislations and regulations be developed with as much incentive as economically justified to motivate market forces. Thus regulatory compliance is required not only to protect public health but also to maintain a viable economy in the control technology sector. Naturally, equitable regulations are necessary.

Regulations, though enabled in a general manner through legislation, can be specifically developed to structure an economic response from particular segments of the economy by an agency. Lobbying efforts can be highly influential in this regard. Currently, the biotechnology industry is without a major trade association or sponsoring government agency, and thus is not able to either effectively lobby or protect its future from the political perspective. Consequently, government research and development can be appropriated to areas that do not benefit the development of biological approaches in pollution control and regulations could reflect an organized lobbies' counter interest.

For example, a frequently cited example is found with biotechnology applications in biomedical product development. Millions of dollars are spent by the National Institutes of Health on the purchase of interferon and to perform research and development on novel production approaches, but few research dollars are allocated to genetic engineering approaches for hazardous waste by the EPA or other agencies. The objective to genetically engineer bacteria, each with the capability to mineralize a specific organic pollutant, would be of major significance to the biotechnology industry in pollution control, as well as industry's waste problem.

As previously mentioned, there is a disparity in professional attitudes between engineers and biologists and these can be highlighted in government bureaucracies.

e. Social

There are several social barriers associated with the use of biological technologies, primarily concerning the ethical nature of genetic engineering and the public health aspects associated with the use of bacteria. Some social opinions reflect the attitude that genetic engineering is counter to the natural course of evolution and is therefore unethical. There is an ethical concern when genetic engineering approaches the manipulation of human genetic structures but is unfounded for the manipulation of primitive bacteria to consume toxic wastes. Finally, there are pathogenic bacteria harmful to public health that are naturally present in the environment, and genetic engineering of hazardous waste consuming organisms will present an insignificant risk in comparison. As hazardous waste is not co-disposed with human sewage, there is little concern for the spread of pathogens by applications of biotechnologies. Nevertheless, there is a continued expression of these concerns, often cited by regulatory officials and design engineers.

3. Scenarios

For this study the author was asked to construct scenarios of future development. Technical, economic, and regulatory factors are considered as the primary variables that will affect the development of biological technologies in the future. Eight scenarios have been constructed from the interaction of these variables to form a basis for projecting the development of the technology. The main factors and their variations are as follows:

Technical Achievements

High	Rapid achievements in the development of cell lines and engineering control techniques based upon industry research and development
Low	Little progress in the development of genetic engineered approaches, low acceptance by the engineering and regulatory community, and few investments in industrial research and development

Economic Developments

Good	Reduction in rate of inflation and finance interest, increase in capital supply and expansion of goods and services, increase in tax base and reduction of deficit, increase in government R&D
Fair	High inflation and interest, decrease in capital supply, slow growth in goods and services, expansion of deficit and decrease in government R&D

Regulatory Compliance

High	Visible industrial effort to mitigate hazardous waste generation, increased public awareness on health concerns, and federal and state enforcement of regulations
Low	Little industrial effort to decrease generation of hazardous waste, low public concerns, and lax federal and state enforcement of regulations

Under a mix of these sets of conditions the following paragraphs present the results of these scenarios and the expected development of biological technologies in hazardous waste control. It should be noted that an individual forecast effort can be highly subjective and a matrix technique as described by Warfield (1972) was used (Table A-4, Appendix). A larger group effort was outside the scope of this study but, if performed, would involve members of the public and private sector (e.g., members of industry, industry trade associations, scientific research establishments, governmental planning organizations, and appropriate government agencies). Large efforts of this nature have been described by Goodman and Lawless (1982) using Delphi techniques, statistical analysis, and subjective appraisals for technology policy development. Nevertheless, considerable market data and professional opinions were collected for this study, and though short of a quantitative econometric forecast model, the qualitative technique described in Table A-4 (Appendix) was felt sufficient to make the forecasts.

Rank	Technical	Economic	Regulatory
1	high	fair	high
2	high	good	high

Though biological technologies can be as capital intensive as alternative approaches, it is felt that in a condition where the overall economy is less than optimum, yet industrial R&D and regulatory compliance remains strong, biotechnologies will be the most prevalent development for the control of hazardous waste. A close second, where the expansion of the market for all hazardous control technologies is greater, is with a good economy; however, in this instance more emphasis would be expected on capital and hardware intensive approaches. In each case a biotechnology market of \$8-11 billion is expected by 1990.

Rank	Technical	Economic	Regulatory
3	high	good	low
4	high	fair	low

Even with low regulatory enforcement over the mid term, it is felt that with a good economy and technical achievements in industrial R&D, continued expansion of the application of biological technology for hazardous waste abatement will result and a market of $7-9 billion in 1990 is expected. Should a sluggish economy with low regulatory compliance occur, yet with continued industrial achievements in R&D, a market of $6-8 billion would be expected in 1990, partially due to an offset of the more capital and energy intensive approaches.

Rank	Technical	Economic	Regulatory
5	low	good	high
6	low	good	low

With a good economy and high regulatory compliance, government R&D is expected to offset investments in the private sector and provide for biological techniques; however, with low achievements and acceptance by the industrial sector, progress would be expected to lag significantly behind alternative approaches. In this case a biotechnology market of $5-7 billion would be expected by 1990. With low regulatory compliance attitudes, the overall market would tend to suffer with only a 2-3 times expansion of the current market to $4-6 billion by 1990.

Rank	Technical	Economic	Regulatory
7	low	fair	high
8	low	fair	low

With high regulatory enforcement yet under diminished technical achievements and a sluggish economy through the 1980's, only modest gains over current biotechnology levels would be expected. The lowest rank occurs for the same situations yet with lax regulatory enforcement and pre-RCRA practices would prevail.

It should be noted that over a seven-year period in the 1970's (1972-1979), there was an overall expansion in pollution abatement expenditures of $7-8 billion 1972 dollars (Rutledge and O'Connor, 1981). Converting these dollars to 1980 dollars (approximately a factor of 2) represents nearly 15 billion dollars for the period. Considering the 1970's focused on conventional pollutants and the 1980's appear to be more directed towards toxic waste treatment and materials recovery under the Resource Conservation and Recovery Act, a biotechnology product market by 1990 of $5-10 billion 1980 dollars should not be considered out of line. These products would supply new treatment applications and replace existing physical/chemical techniques. It assumes that technical achievements in biotechnology applications will be made.

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APPENDIX

Table A-1

COMPANIES SUPPLYING PRODUCTS FOR HAZARDOUS WASTE TREATMENT APPLICATION

A. Advanced Waste Treatment Processes

Air Products & Chemicals, Inc.
Andco Environmental Processes, Inc.
Astro Metallurgical Corp.
Atara, Inc.
Autotrol Corp., Bio-Systems Div.
BIF, a Unit of General Signal
BINAB USA, Inc.
Calgon Corp.
Capital Controls Co.
Ralph B. Carter Co.
Celanese Chemical Co., Inc.
Clow Corp.
Crane Co.
Dorr-Oliver Inc.
Du Pont Co., Environmental Products
ECODYNE/Smith & Loveless Div.
Ecolotrol Corp.
Eco-Research Ltd.
Eimco PMD Div., Envirotech Corp.
Envirex Inc., a Rexnord Co.
Environment/One Corp.
Environmental Conditioners, Inc.
Environmental Elements Corp.
Environmental Systems Div.,
  George A. Hormel & Co.
Euramca Inc.
Fluid Systems Div., UOP, Inc.
B.F. Goodrich General Products Co.
Hercules, Inc.
High Voltage Engineering Corp.
Hydro-Aerobics International, Inc.
Hydro Clear Corp.
Industrial Filter & Pump Mfg. Co.
Infilco Degremont, Inc.
Kenics Corp.
Lakeside Equipment Corp.
LYCO
Met-Pro Systems, Inc.
Mixing Equipment Co., Inc.
Munters Corp.

Table A-1 (Cont.)

COMPANIES SUPPLYING PRODUCTS FOR HAZARDOUS WASTE TREATMENT APPLICATION

A. Advanced Waste Treatment Processes (Cont.)

National Hydro Systems, Inc.
Nelson Industrial Services, Inc.
Neptune Microfloc, Inc.
Northrop Services, Inc.
PCI Ozone Corp.
Passavant Corp.
Pentech Div., Houdaille Industries, Inc.
Permutit Co.
Pollution Control, Inc.
Polybac Corp.
Sanitaire-Water Pollution Control Corp.
TLB Corp.
Technological Resources, Inc.
Telecommunications Industries, Inc.
Turbitrol Co.
Van Nostrand Reinhold
Walker Process Corp.
Wallace & Tiernan Div., Pennwalt Corp.
Western Dynetics
Zimpro Inc.

B. Digestion Tank Equipment

Aquatech Inc.
Atara, Inc.
C-E Bauer
Ralph B. Carter Co.
Clow Corp.
Dorr-Oliver Inc.
Dresser Industries, Jeffrey Mfg. Div.
Eco-Research, Ltd.
Eimco PMD Div., Envirotech Corp.
Envirex Inc., a Rexnord Co.
Euramca Inc.
FMC Corp.
Fiberglass Specialty Co.
Gorman-Rupp Co.
Keene Corp.
Lakeside Equipment Corp.
National Hydro Systems, Inc.
National Power Rodding Corp.
Peabody Welles
Pollution Control, Inc.
Sanitaire-Water Pollution Control Corp.
TLB Corp.
Vaughan Co., Inc.
Walker Process Corp.

Table A-1 (Cont.)

COMPANIES SUPPLYING PRODUCTS FOR HAZARDOUS WASTE TREATMENT APPLICATION

C. Oxygen Activated Sludge Systems

Aeration Industries, Inc.
Air Products & Chemicals, Inc.
Ralph B. Carter Co.
Dorr-Oliver Inc.
EDC/Enviro Development Co., Inc.
Ecolotrol, Inc.
Eimco PMD Div., Envirotech Corp.
FMC Corp.
Howe-Baker Engineers
Hydro-Aerobics International, Inc.
Keene Corp.
Kenics Corp.
Pentech Div., Houdaille Industries, Inc.
Union Carbide Corp., Environmental Systems
Zimpro Inc.

D. Irrigation Systems

Exidyne, Inc.
Lindsay Mfg. Co.
Magney Electric Motors, Inc.
Technological Resources, Inc.
Washington Aluminum Co., Inc.

E. Pharmaceutical Equipment Suppliers

Table A-2

CALCULATION OF COST COMPLIANCE TO INTERIM STATUS STANDARDS FOR AVERAGE SURFACE IMPOUNDMENT

1.) Data Provided in EPA (1980)

- 22 industries discharging 13.7 million metric tons (MT)
- $510 million annual cost of compliance for all RCRA interim status standards
- 50% of this cost for surface impoundments
- 70% of this cost for engineering compliance
- calculation of cost per surface impoundment
  - -7000 impoundments as RCRA facilities
  - -50 million MT generated annually (all industries)

a) $$\frac{13.7\text{ million MT}}{50\text{ million MT}} \times 7000\text{ impoundments} = 1918\text{ impoundments probable in EPA survey}$$

b) $$\frac{\$510\text{ million} \times 50\% \times 70\%}{1918\text{ surface impoundments}} = \$93{,}065\text{ per ave. impoundment}$$

2.) Data Provided in SCS (1979)

- upgrading of wastewater treatment impoundment of 50 acres @ flow of 5000 cubic meters (1.45 million gallons) per day
- capital of $10,655,000/20 years; 1st year operating $22,000

a) $$\frac{\$10.7\text{ million}}{20\text{ yr}} + \$22{,}000 = \$554{,}000/\text{yr}$$

SOURCE: EPA, Regulatory Analysis of the Resource Conservation and Recovery Act, Subtitle C., PB 81-181471, April 1980

SCS, Assessment of Petroleum Industry Cost of Compliance with Proposed Hazardous Waste Regulations, for American Petroleum Institute, April 1979

Table A-3

SAMPLE CALCULATION OF PRODUCT GROWTH PROJECTION FOR BIOTECHNOLOGIES (1980 DOLLARS) IN TABLE V-5 OF TEXT

1.) 1982 Projected Rate

Pollution Abatement Sector	$	Range given in Table V-5.
lagoon (impoundment) retrofit	70 million	
on-site treatment		
solids (e.g. landfarms)*	75 million	
wastewater facilities**	387 million	
off-site treatment	10 million	
	542 million	355-715 millio

2.) 1986 Projection

40% annual growth compounded 4 years = 3.84

$542 million x 3.84 = $2081 million 1235-2565 millic

3.) 1990 Projection

20% annual growth compounded 4 years = 2.07

$2.08 billion x 2.07 = $4.30 billion 2.38-5.15 billic

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*Capital expenditures are estimated to be less than one-third of the on-site capital and operating biological treatment costs of solids presented in Figure V-1 of text.

**Value represents 30% of wastewater capital expenditures given in Table V-3 of text.

Table A-4

METHOD FOR DEVELOPING SCENARIO RANKINGS AND CALCULATING RELATED BIOTECHNOLOGY PRODUCT EXPENDITURES FOR 1990

A. RANKING METHOD

Forecast Variables	Weighting Factors
Technical Achievements*	
High	4
Low	1
Economic Developments	
Good	3
Fair	2
Regulatory Compliance	
High	3
Low	2

		Technical Achievement			
		High (4)		Low (1)	
		Economic Development		Economic Development	
		Good (3)	Fair (2)	Good (3)	Fair (2)
Regulatory Compliance	High (3)	(4) (3) (3) 36	(4) (2) (3) 24	(1) (3) (3) 9	(1) (2) (3) 6
	Low (2)	(4) (3) (2) 24	(4) (2) (2) 16	(1) (3) (2) 6	(1) (2) (2) 4

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*Technical achievements are provided the major weight. Without technical achievements, no technology could be applied effectively.

Table A-4 (Continued)

B. RANK

1.) From Matrix

	Rank	Score	Technical	Economic	Regulatory
	1.	36	high	good	high
**	*2.	24	high	good	low
	*3.	24	high	fair	high
	4.	16	high	fair	low
	5.	9	low	good	high
	*6.	6	low	good	low
	*7.	6	low	fair	high
	8.	4	low	fair	low

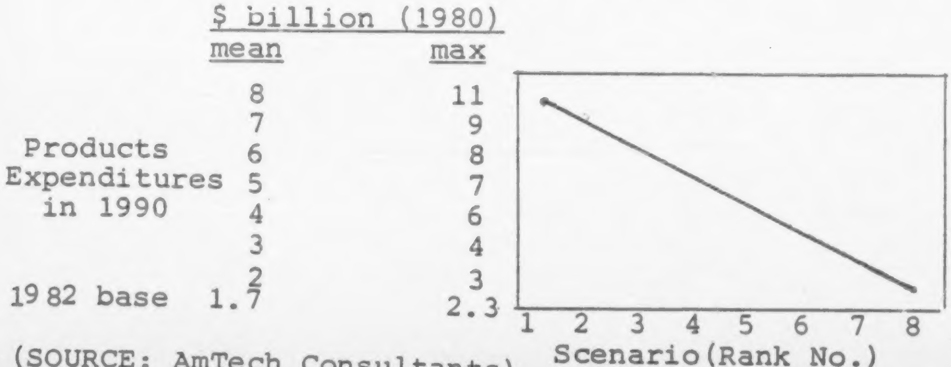
*,** Subjective adjustments
*though given equal weights, economic developments outweigh regulatory compliance in the event of a tie

**the interaction of high-fair-high is felt to be more favorable for biological approaches than high-good-high. Otherwise, the first rank column would apply.

2.) Adjusted Rank

Rank	Technical	Economic	Regulatory
1.	high	fair	high
2.	high	good	high
3.	high	good	low
4.	high	fair	low
5.	low	good	high
6.	low	good	low
7.	low	fair	high
8.	low	fair	low

C. STRATIFICATION OF PRODUCT EXPENDITURES



This distribution is a linear stratificati of product markets presented in Table V- from $8-11 billion (1990 projection, whe $8 billion is the mea of the given range) down to the 1982 base of $1.7-2.3 billion.

(SOURCE: AmTech Consultants)